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NEW UTILITY PATENT APPLICATION TRANSMITTAL
(Large Entity)

(Only for new nonprovisional applications under 37 CFR 1.53(b))

Docket No.
1038-765 MIS

Total Pages in this Submission
3

JC380 U.S. PTO 01/14/98

TO THE ASSISTANT COMMISSIONER FOR PATENTS
Box Patent Application
Washington, D.C. 20231

Transmitted herewith for filing under 35 U.S.C. 111(a) and 37 C.F.R. 1.53(b) is a new utility patent application for an invention entitled:

CHIMERIC ANTIBODIES FOR DELIVERY OF ANTIGENS TO SELECTED CELLS OF THE IMMUNE SYSTEM

and invented by:

Naveen N. Anand, Brian H. Barber, George A. Cates, Judith E. Caterini and Michel H. Klein

If a CONTINUATION APPLICATION, *check appropriate box and supply the requisite information:*

☒ **Continuation** ☐ **Divisional** ☐ **Continuation-in-part (CIP)** of prior application No.: **08/483,576**

Enclosed are:

Application Elements

1. ☒ Filing fee as calculated and transmitted as described below
2. ☒ Specification having 44 pages and including the following:
   a. ☒ Descriptive Title of the Invention
   b. ☒ Cross References to Related Applications *(if applicable)*
   c. ☐ Statement Regarding Federally-sponsored Research/Development *(if applicable)*
   d. ☐ Reference to Microfiche Appendix *(if applicable)*
   e. ☒ Background of the Invention
   f. ☒ Brief Summary of the Invention
   g. ☒ Brief Description of the Drawings *(if drawings filed)*
   h. ☒ Detailed Description
   i. ☒ Claim(s) as Classified Below
   j. ☒ Abstract of the Disclosure
3. ☒ Drawing(s) *(when necessary as prescribed by 35 USC 113)*
   a. ☐ Formal
   b. ☒ Informal
   Number of Sheets 10

NEW UTILITY PATENT APPLICATION TRANSMITTAL
(Large Entity)

(Only for new nonprovisional applications under 37 CFR 1.53(b))

Docket No. 1038-765 MIS
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Application Elements (Continued)

4. ☒ Oath or Declaration
   a. ☐ Newly executed *(original or copy)* ☐ Unexecuted
   b. ☒ Copy from a prior application (37 CFR 1.63(d)) *(for continuation/divisional application only)*
   c. ☒ With Power of Attorney ☐ Without Power of Attorney

5. ☒ Incorporation By Reference *(usable if Box 4b is checked)*
   The entire disclosure of the prior application, from which a copy of the oath or declaration is supplied under Box 4b, is considered as being part of the disclosure of the accompanying application and is hereby incorporated by reference therein.

6. ☐ Computer Program in Microfiche *(Appendix)*

7. ☒ Nucleotide and/or Amino Acid Sequence Submission *(if applicable, all must be included)*
   a. ☒ Paper Copy
   b. ☒ Computer Readable Copy *(identical to computer copy)*
   c. ☒ Statement Verifying Identical Paper and Computer Readable Copy

Accompanying Application Parts

8. ☐ Assignment Papers *(cover sheet & document(s))*

9. ☐ 37 CFR 3.73(B) Statement *(when there is an assignee)*

10. ☐ English Translation Document *(if applicable)*

11. ☒ Information Disclosure Statement/PTO-1449 ☐ Copies of IDS Citations

12. ☒ Preliminary Amendment

13. ☒ Acknowledgment postcard

14. ☐ Certificate of Mailing
    ☐ First Class ☐ Express Mail *(Specify Label No.):* _____

15. ☐ Certified Copy of Priority Document(s) *(if foreign priority is claimed)*

NEW UTILITY PATENT APPLICATION TRANSMITTAL
(Large Entity)

(Only for new nonprovisional applications under 37 CFR 1.53(b))

Docket No.
1038-765 MIS

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3

Accompanying Application Parts (Continued)

16. ☐ Additional Enclosures *(please identify below):*

Fee Calculation and Transmittal

CLAIMS AS FILED

For	#Filed	#Allowed	#Extra		Rate	Fee
Total Claims	33	- 20 =	13	x	$22.00	$286.00
Indep. Claims	9	- 3 =	6	x	$82.00	$492.00
Multiple Dependent Claims (check if applicable) ☐						$0.00
					BASIC FEE	$790.00
OTHER FEE *(specify purpose)* _____						$0.00
					TOTAL FILING FEE	$1,568.00

☒ A check in the amount of **$1,568.00** to cover the filing fee is enclosed.

☐ The Commissioner is hereby authorized to charge and credit Deposit Account No. as described below. A duplicate copy of this sheet is enclosed.

- ☐ Charge the amount of as filing fee.
- ☐ Credit any overpayment.
- ☐ Charge any additional filing fees required under 37 C.F.R. 1.16 and 1.17.
- ☐ Charge the issue fee set in 37 C.F.R. 1.18 at the mailing of the Notice of Allowance, pursuant to 37 C.F.R. 1.311(b).

Dated: **January 12, 1998**

Signature

M.I. Stewart,
Registration No. 24,973

cc:

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Our Ref: 1038-765 MIS:as

In re patent application

No.

Applicant: Naveen N. Anand et al

Title: CHIMERIC ANTIBODIES FOR DELIVERY OF ANTIGENS TO SELECTED CELLS OF THE IMMUNE SYSTEM

Filed:

Group No.

Examiner:

January 13, 1998

PRELIMINARY AMENDMENT

BY COURIER

The Commissioner of Patents
and Trademarks,
Box Patent Application,
Washington, D.C. 20231,
U.S.A.

Dear Sir:

Please amend this application in the following manner:

In the Disclosure:

On page 6, line 16, insert "97,202" after "No.";

On page 10, line 11, insert ", comprising panels A and B," after "Figure 6"; line 22, insert ", comprising panels A and B," after "Figure 9"; line 23, insert the word "panel" before "A)"; and "B)"; line 25, insert the word "panel" before "A)"; line 26, insert "panel" before "B)"; line 27, insert ", comprising panels A and B," after "Figure 10"; line 29, insert "panel" before "A)"; line 30, insert "panel" before "B)".

Page 19, line 18, insert "97,202" after "No." and insert "June 23, 1995" after "on".

Insert the hard copy of the Sequence Listing after the last page of disclosure and immediately preceding the claims.

In the Claims:

Amend claims 1 and 27 as follows:

1. (Amended) A recombinant conjugate antibody molecule, consisting of a bivalent monoclonal antibody moiety having heavy and light chains and specific for a surface structure of antigen presenting cells genetically modified to contain at least one antigen moiety exclusively at at least one preselected site on said monoclonal antibody moiety, whereby said conjugate antibody molecule is capable of delivering said antigen moiety to the antigen presenting cells of a host and capable of eliciting an immune response to said antigen moiety in the host.

27. (Amended) An immunogenic composition, comprising, as an active component thereof, a conjugate antibody molecule consisting of [comprising] a bivalent monoclonal antibody moiety having heavy and light chains and specific for a surface structure of antigen presenting cells genetically modified to contain at least one antigen moiety exclusively at at least one preselected site on said monoclonal antibody moiety exclusively at at least one preselected site on said monoclonal antibody moiety, whereby said conjugate antibody molecule is capable of delivering said antigen moiety to the antigen presenting cells of a host and capable of eliciting an immune response to said antigen moiety in the host.

Cancel claims 26 and 29 to 33.

In the Drawings:

It is proposed to Amend Figures 1, 2, 3 and 4 as shown on the enclosed print in red.

REMARKS

In an Advisory Action on the parent application, the Examiner indicated that the Amendment submitted September 24, 1997 would not be entered in the parent filing, on the basis that:

> "The proposed amendments to the claims would require an additional art search and application of any art that is found".

While not agreeing with the Examiner in this regard, this Continuation Application is being submitted with revisions to the claims corresponding to those proposed to be made in the parent application, so that the Examiner can properly consider the same.

In addition, the Examiner indicated in the Advisory Action that the IDS was not considered. The IDS is being re-submitted with this Continuation Application so that the contents may be considered by the Examiner. This new PTO-1449 also includes a listing of the references referred to in the disclosure. Copies of some of the references were provided in the parent filing. The missing copies will follow shortly.

In the Final Action in the parent application, the Examiner rejected claims 1 to 11 under 35 USC 112, second paragraph, as being indefinite, in view of the use of "consisting essentially of" in claim 1. The Examiner's suggestion to employ "consisting of" in claim 1 has been adopted. The same language is used in claim 27. It is submitted that claims 1 to 11 are no longer open to rejection under 35 USC 112, first paragraph.

In the Final Action on the parent application, the Examiner rejected claims 1 to 11 and 27 to 28 under 35 USC 102(a) as being anticipated by Baier et al.

The applicants' claims are directed to a recombinant conjugate antibody molecule which consists of a monoclonal antibody moiety specific for a surface structure of antigen presenting cells genetically modified to contain at least one antigen moiety exclusively at at least one preselected site on the monoclonal antibody, whereby the conjugate antibody molecule is capable of delivering the antigen moiety to the antigen presenting cells of a host and capable of eliciting an immune response to the antigen moiety. Applicants claims include immunogenic compositions comprising such molecule.

As previously pointed out, Baier et al teach the provision of a gp-120 peptide and antibody Fab fragments

reactive with surface structures displayed on APCs. Such fusions are generated by DNA methodology. It is clear that the reference describes only the use of antibody Fab fragments in the chimeric molecules. The Examiner asserted in the Final Action on the parent application that applicants terminology "monoclonal antibody moiety" includes the Fab fragments of Baier.

It has previously been pointed out to the Examiner in the parent case that applicants employed a complete monoclonal antibody moiety. The Examiner asserts, however, that such limitation is not found in the claims. While not necessarily agreeing with the Examiner, the monoclonal antibody moiety now is defined in claims 1 and 2 as "bivalent". That the monoclonal antibody is bivalent is evident from consideration of applicants subsidiary claims. In this regard, it is noted that claim 3, for example, refers to "the heavy and light chains of said monoclonal antibody moiety". Accordingly, the monoclonal antibody moiety of claim 1 (said monoclonal antibody moiety) must contain both heavy and light chains and hence is bivalent. For further emphasis and to provide antecedent basis for the language of claim 3, claim 1 has been additionally amended to refer to the monoclonal antibody moiety possessing heavy and light chains.

It is submitted that the "monoclonal antibody moiety" recited in claim 1 is not anticipated by the Fab fragments disclosed by Baier et al. Even if the term were interpreted in the manner of the Final Action in the parent application, and thereby claim 1 arguably anticipated, it is abundantly clear that claims 3 to 11 cannot be considered anticipated since those claims specifically require that the monoclonal antibody moiety have both heavy and light chains, which clearly is not the case for the monovalent Fab fragment described by Baier et al.

Having regard to the revisions made to claim 1 and the above discussion, it is submitted that each of claims 1 to

11 and 27 to 28 is not anticipated by Baier et al and hence claims 1 to 11, 27 and 28 are not open to rejection under 35 USC 102(a) as being anticipated by Baier et al.

In the Final Action on the parent application, the Examiner rejected claims 1 to 11, 27 and 28 under 35 USC 102(b) as being anticipated by or, in the alternative, under 35 USC 103(a) as being obvious over Barber et al U.S. Patent 4,950,480.

As previously noted in the prosecution of the parent application, the Barber et al reference is acknowledged in the specification, for example, on page 2, line 36 to page 3, line 10. As described therein, biotin-streptavidin based interaction was used to link antibody and antigen to provide a molecule used for targeting the antigen to the antigen-presenting cells. There are inherent disadvantages to the chemical coupling technique employed by Barber et al, such as yield (typically about 20%) and the variability between different preparations. There is no adequate control on the amount of coupled peptide, as well as the exact location of the reaction. Purification is usually required and losses of material can be significant. These disadvantages of the Barber et al system are echoed in the Baier reference in the paragraph bridging pages 2357 and 2358.

The present invention meets the need to produce conjugates of targeting antibodies and antigens of specific reproducible structure in high yields. In the present invention, as defined in claim 1, there is provided a <u>recombinant</u> conjugate antibody molecule which consists of a bivalent monoclonal antibody moiety having heavy and light chains and specific for a surface structure of antigen presenting cells, <u>genetically modified</u> to contain at least one antigen moiety <u>exclusively at at least one preselected site</u> on the monoclonal antibody moiety. This language defines a structure different from that provided by Barber et al.

In the Final Action on the parent application, the Examiner states:

> "Examiner agrees that the process by which the antibody-antigen fusion proteins were generated are different. However, product by process is not patentably distinct in a product claim. Applicant is claiming antibody-antigen fusion proteins. The process by which the fusion proteins were generated is irrelevant. In order to demonstrate patentability, applicant must show that the recombinant antigen-antibody fusion proteins have properties which differ from the chemically conjugated fusion proteins." (Emphasis added).

Applicant agrees largely with this analysis. However, applicants claims recite structural limitations which distinguish the claimed product from those described in Barber et al. Applicants claims recite that the monoclonal antibody moiety has been genetically modified to contain the antigen moiety exclusively at at least one preselected site on the monoclonal antibody moiety. This structural limitation is achievable only by recombinant methodology and hence the recitation of a recombinant conjugate antibody molecule also imparts a structural limitation on the molecule which differentiates the structure from that obtained in Barber et al.

The lack of adequate control on the amount of coupled antigen as well as the exact location of the reaction in the prior art of Barber et al ensures that the antigen moiety is not coupled exclusively at at least one preselected site into the monoclonal antibody moiety as required by applicants claims.

The Examiner further stated in the Final Action on the parent file that:

> "... applicant argues the disadvantages in the chemical coupling technique. However, as applicant is claiming product claims, the advantages or disadvantages of the different methods used to obtain the product are irrelevant."

However, as explained above, it is these very disadvantages of the method used which leads to the different structural features of claim 1 which differentiate from the cited prior art. By effecting the recombinant production claimed therein, it is possible to locate the antigen moiety exclusively at a preselected site on the antibody moiety, a result which is not obtainable using the biotin-streptavidin based interaction to link the antibody and antigen as in the case of the Barber et al U.S. Patent No. 4,950,480 reference.

Accordingly, it is submitted that claims 1 to 11 and 27 to 28 are not open to rejection under 35 USC 102(b) as anticipated by, or in the alternative, under 35 USC 103(a) as obvious over U.S. Patent No. 4,950,480.

In the parent application, the Examiner rejected claims 1 to 11, 27 and 28 under 35 USC 103(a) as being unpatentable over U.S. Patent No. 4,950,480 in view of U.S. Patent No. 5,196,520.

The Examiner, accordingly, made a third rejection of claims 1 to 11, 27 and 28 based on U.S. Patent No. 4,950,480:

(A) anticipated by 4,950,480

(B) obvious over 4,950,480 alone

(C) obvious over 4,950,480 in combination with 5,196,320.

The necessity to make three rejections of the same claims shows a clear lack of conviction on the part of the Examiner as to the relationship of the claims to the prior art.

The Examiner stated in the Final Action on the parent application that the '320 patent:

> "teaches the generation of single chain antibody fusion proteins. As the chains recite a monoclonal antibody moiety, the term would include single chain antibodies."

Applicants have discussed the Examiner's interpretation of the applicant's claim language above with respect to the rejection based on Baier et al. Applicant's claims now specifically recite that the monoclonal antibody has heavy and light

chains, thereby clearly excluding the single chain antibody fusions such as are disclosed in the '320 patent.

Having regard to the applicant's claim language and having regard to the clear distinctions between applicants conjugate molecules and those disclosed in the '480 patent, it is submitted that the claims of this application are patentably distinguished from the combination of U.S. Patents Nos. 4,950,480 and 5,196,320 and hence claims 1 to 11 and 27 to 28 are not open to rejection under 35 USC 103 as unpatentable over the combination of prior art.

These amendments and arguments in support of patentability of the amended claims are submitted at this time in the interests of expediting prosecution.

It is proposed to amend the drawings and the description thereof in order to separately label the views contained within the Figures and in accordance with the description of the views of Figures 1 to 4 as contained on page 9, line 4 to page 10, line 3. In addition, the disclosure has been amended in order to indicate that Figures 6, 9 and 10 include multiple panels and to provide further identification of the panels. These changes correspond to those made in the parent filing.

It is believed that this application is now in condition for allowance and early and favourable consideration and allowance are respectfully solicited.

Respectfully submitted,

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TITLE OF INVENTION

CHIMERIC ANTIBODIES FOR DELIVERY OF ANTIGENS TO SELECTED CELLS OF THE IMMUNE SYSTEM

FIELD OF INVENTION

The present invention is concerned with novel recombinant antibody molecules genetically modified to contain an antigen moiety for the purpose of delivery of the antigen moiety to antigen-presenting cells of the immune system.

BACKGROUND OF INVENTION

Current theories of immunology suggest that, in order to provide a potent antibody response, an antigen must be seen by both B cells, which subsequently develop into the antibody producing cells, and also by helper T-cells, which provide growth and differentiation signals to the antigen specific B-cells. Helper T-cells recognize the antigen on the surface of antigen-presenting cells (APC) in association with Class II major histocompatibility complex (MHC) gene products.

There are significant advantages in using proteins and peptides derived from proteins of infectious organisms as part of subunit vaccines. The search for such suitable subunits constitutes a very active area of both present and past research. Advances in techniques of recombinant DNA manipulations, protein purification, peptide synthesis and cellular immunology have greatly assisted in this endeavour. However, a major stumbling block to the use of such materials as vaccines has been the relatively poor *in-vivo* immunogenicity of most protein subunits and peptides. Generally, the immune response to vaccine preparations is enhanced by the use of adjuvants. However, the only currently licensed adjuvants for use in humans are aluminum hydroxide and aluminum phosphate, collectively termed alum, which is limited in its effectiveness as a potent adjuvant. There is thus a need for new adjuvants with the desired

efficacy and safety profiles.

Several adjuvants, such as Freund's Complete Adjuvant (FCA), syntex and QS21, have been used widely in animals (ref 1 - Throughout this application, various references are referred to in parenthesis to more fully describe the state of the art to which this invention pertains. Full bibliographic information for each citation is found at the end of the specification, immediately preceding the claims. The disclosures of these references are hereby incorporated by reference into the present disclosure). In animals, administration of peptides and protein antigens with these adjuvants, has been shown to result in neutralizing antibodies against a variety of infectious organisms (refs. 3 to 8). A novel way of engaging both the B and T cell components of an immune response has been described, which uses anti-class II monoclonal antibodies (mabs) coupled to antigens to target class II bearing antigen presenting cells (APC's) (refs 9 to 11, also U.S. Patents Nos. 5,194,254 and 4,950,480 assigned to the assignee hereof). Experiments carried out *in-vivo* in rodents and rabbits using this technology, (refs. 9 to 12), have demonstrated convincing proof of enhancement in immunogenicity of antigens, in the absence of conventional adjuvants. Several research groups have used other cell surface markers such as Surface Immunoglobulin (sIg) (ref. 13), Fc γ receptors, CD45 and MHC class I (refs. 14 to 17), to achieve targeting to APC's; however, most of these latter studies involve *in-vitro* experiments and lack animal data. Another group of studies reports the use of antibodies of irrelevant specificity to carry antigen epitopes (refs. 18 to 24). The *in-vivo* studies utilizing such "antigenized antibodies", however, involves the use of conventional adjuvants and some of them require multiple injections for the desired effect (ref. 24).

In previous studies using anti-class II mab as a

targeting molecule (refs. 9 to 11), biotin-streptavidin based interaction was used to link the antibody and antigen. There are some inherent disadvantages with such chemical coupling techniques, such as yields (about 20%) and also the variability factor between different preparations. There is also no adequate control on the amounts of coupled peptide as well as the exact location of the reaction. Additionally, further purification is usually required and, therefore, losses in material can be significant.

Recently a study reporting *in-vitro* data using anti-human class II Fab-peptide fusions generated by recombinant DNA methodology, has been published (ref. 27). There are several differences between these fusions and the present invention in that the former is an *E. coli* expressed monovalent protein fragment of a divalent whole immunoglobulin molecule and also is an *in-vitro* study. The common problems encountered in bacterial expression systems include expression as inclusion bodies which require solubilization and refolding with extensive product losses. The expression of whole antibody is presently not possible in *E. coli* and, therefore, the monovalent Fab may not have the requisite affinity for *in-vivo* targeting. There are, thus, several advantages in using a whole IgG recombinant system as described herein.

There remains a need, therefore, to produce conjugates of targeting antibodies and antigens of specific reproducible structure in high yields. Such conjugate antibody molecules and nucleic acid molecules encoding the same are useful in immunogenic preparations including vaccines, for protection against disease caused by a selected pathogen and for use as and for the generation of diagnostic reagents and kits.

SUMMARY OF INVENTION

The present invention includes novel recombinant

conjugate antibody molecules which have been genetically modified to contain an antigen moiety for delivery of the antigen moiety to antigen-presenting cells of the immune systems.

Accordingly, in one aspect of the present invention, there is provided a conjugate antibody molecule, comprising a monoclonal antibody moiety specific for a surface structure of antigen-presenting cells genetically modified to contain at least one antigen moiety exclusively at at least one preselected site in the monoclonal antibody. The conjugate antibody molecule is capable of delivering the antigen moiety to the antigen presenting cells of a host and capable of eliciting an immune response to the antigen moiety in the host.

Genetically modifying the antibody moiety to contain the antigen moiety only at preselected sites ensures that a product with consistent composition and structure is obtained.

The antigen presenting cells may be any convenient antigen-presenting cells of the immune system, including class I or class II major histocompatibility expressing cells (MHC), B-cells, T-cells or professional antigen-presenting cells including dendritic cells, and $CD4^+$ cells.

The at least one antigen moiety preferably is located at at least one end of at least one of the heavy and light chain of the monoclonal antibody moiety, particularly the C-terminal end of both the heavy and light chain. The at least one antigen moiety is preferably directly linked with the C-terminal end of both the heavy and light chains of the monoclonal antibody moiety.

One feature of the present invention is the ability to obtain an enhanced immune response to an antigen without the use of an adjuvant. Accordingly, in one

embodiment of the invention, the at least one antigenic moiety may comprise an inherently weakly-immunogenic antigen moiety. The at least one antigen moiety may comprise a plurality of antigen moieties, which may be the same or different. In addition, the at least one antigen moiety may be a peptide having 6 to 100 amino acids and containing at least one epitope.

The novel conjugate antibody molecules provided herein are produced by recombinant procedures which include the provision of novel nucleic acid molecules and vectors containing the same.

In accordance with another aspect of the present invention, there is provided a nucleic acid molecule comprising a first nucleotide sequence encoding a chain of a monoclonal antibody specific for a surface structure of antigen-presenting cells selected from the group consisting of the heavy chain and the light chain of the monoclonal antibody, a second nucleotide sequence encoding at least one antigen and a third nucleotide sequence comprising a promoter for eukaryotic cell expression of a fusion protein comprising said monoclonal antibody chain and said at least one antigen. The antigen presenting cells may be any of those described above.

The first nucleotide sequence and the second nucleotide sequence are preferably directly linked in a single transcriptional unit under control of the promoter. The third nucleotide sequence preferably is disposed at the 5′-end of the first nucleotide sequence.

The present invention further includes vectors comprising the nucleic acid molecules provided herein. In one specific embodiment of this aspect of the invention, this vector may contain a first nucleic acid molecule comprising a first nucleotide sequence encoding the heavy chain of a monoclonal antibody specific for a surface structure of antigen-presenting cells, a second

nucleotide sequence encoding at least one antigen and a third nucleotide sequence comprising a promoter for eukaryotic cell expression of a fusion protein comprising said monoclonal antibody heavy chain and said at least one antigen as a first transcriptional unit, and a second nucleic acid molecule comprising a first nucleotide sequence encoding the light chain of a monoclonal antibody specific for a surface structure of antigen-presenting cells, a second nucleotide sequence encoding at least one antigen and a third nucleotide sequence comprising a promoter for eukaryotic cell expression of a fusion protein comprising said monoclonal antibody light chain and said at least one antigen as a second transcriptional unit.

One particular vector has the characteristics of plasmid pCMVdhfr.chLCHC (ATCC Accession No.).

The production of the conjugate antibody molecule comprising a monoclonal antibody moiety specific for a surface structure of antigen-presenting cells and at least one antigen moiety in mammalian cells constitutes a further aspect of the invention. Such procedure comprises:

constructing a first nucleic acid molecule containing a first nucleotide sequence encoding a heavy chain of said monoclonal antibody and a second nucleotide sequence encoding at least one antigen,

constructing a second nucleotide acid molecule containing a first nucleotide sequence encoding a light chain of said monoclonal antibody and a second nucleotide sequence encoding said at least one antigen, and

coexpressing said first and second nucleic acid molecules in mammalian cells to form said conjugate antibody molecule.

The coexpression of the first and second nucleic acid molecules includes constructing an expression vector containing the first and second nucleic acid molecules as

independent transcriptional units, which preferably also contain a promoter operable in mammalian cells to direct the coexpression. The coexpression includes secretion of the conjugate molecule and the conjugate molecules may be separated from the culture medium and purified, preferably by binding to protein A and selectively eluting the conjugate molecules.

A further aspect of the invention provides an immunogenic composition comprising a conjugate antibody molecule as provided herein or a nucleic acid molecule as provided herein. The immunogenic composition preferably is formulated as a vaccine for *in vivo* administration to a host to elicit an immune response against disease(s) caused by a pathogen producing the at least one antigen.

According to an additional aspect of the invention, there is provided a method of generating an immune response in a host, comprising administering thereto an immunoeffective amount of a immunogenic composition as provided herein.

The novel conjugate antibody molecules provided herein also are useful in diagnostic applications. Accordingly, in yet a further aspect of the invention, there is provided a method of determining the presence of a selected antigen in a sample, which comprises:

(a) immunizing a host with a conjugate antibody molecule as provided herein, wherein the at least one antigen moiety is said selected antigen to produce antibodies specific to the selected antigen;

(b) isolating the antibodies;

(c) contacting the sample with the isolated antibodies to produce complexes comprising any selected antigen in the sample and the selected antigen-specific antibodies; and

(d) determining production of the complexes.

The invention further comprises a diagnostic kit for determining the presence of a selected antigen in a

sample, comprising:

(a) a conjugate antibody molecule as provided herein, wherein the at least one antigen moiety is the selected antigen;

(b) means for detecting the production of complexes comprising any selected antigen in the sample and selected antigen-specific antibodies to said selected antigen; and

(c) means for determining production of the complexes.

The invention further includes methods for producing antibodies specific for a selected antigen. One such procedure comprises:

(a) immunizing a host with an effective amount of an immunogenic composition as provided herein, wherein the at least one antigen is a selected antigen to produce antibodies specific for the selected antigen; and

(b) isolating the antibodies from the host.

Another such procedure comprises:

(a) administering an immunogenic composition as provided herein, wherein said at least one antigen is a selected antigen, to at least one mouse to produce at least one immunized mouse;

(b) removing B-lymphocytes from the at least one immunized mouse;

(c) fusing the B-lymphocytes from the at least one immunized mouse with myeloma cells, thereby producing hybridomas;

(d) cloning the hybridomas;

(e) selecting clones which produce anti-selected antigen antibody;

(f) culturing the anti-selected antigen antibody-producing clones; and then

(g) isolating anti-selected antigen antibodies from the cultures.

BRIEF DESCRIPTION OF DRAWINGS

The invention is described in more detail herein with reference to the accompanying drawings, in which:

Figure 1A shows the DNA sequence (SEQ ID No: 1) and derived amino acid sequence (SEQ ID No: 2) of the variable region of murine 44H104 mab light chain. The sequence of the peptide mediating secretion is shown in italicized script.

Figure 1B shows the DNA sequence (SEQ ID No: 3) and derived amino acid sequence (SEQ ID No: 4) of the variable region of murine 44H104 mab heavy chain. The sequence of the secretory peptide mediating secretion is shown in italicized script.

Figure 2A shows the amino acid sequence (SEQ ID No: 5), in single letter code of peptide CTLB36, and nucleotide sequence encoding the same (SEQ ID No: 6), including two termination codons.

Figure 2B shows a scheme for construction and assembly of a gene coding for CTLB36 using overlap extension PCR.

Figure 2C shows synthetic polynucleotides CTLB 36.1, CTLB 36.2 and CTLB 36.3 and their sequences (SEQ ID Nos: 7, 8 and 9) used in the scheme of Figure 2B and primers LC.F, HC.F and R and their sequences (SEQ ID Nos: 10, 11 and 12) used in the PCR reaction.

Figure 3A shows a scheme for construction of 44H104 light chain gene using PCR-generated DNA cassettes V_L and C_L.

Figure 3B shows the oligonucleotide primers Pr. 1, Pr. 2, Pr. 3 and Pr. 4 (SEQ ID Nos: 13, 14, 15 and 16) synthesized for PCR reactions to obtain V_L and C_L gene cassettes.

Figure 4A shows a scheme for construction of chimeric 44H104 heavy chain gene using PCR-generated V_H and C_H DNA cassettes.

Figure 4B shows the oligonucleotide primers Pr. 5,

Pr. 6, Pr. 7 and Pr. 8 (SEQ ID Nos: 17, 18, 19 and 20) synthesized for PCR reactions to obtain V_H and C_H gene cassettes.

Figure 5 contains the structures and schemes for construction of pRc/CMV based expression vectors for genes encoding chimeric light and heavy chain fusions with CLTB36. Plasmid pCMV·chLCHC is a tandem co-linear construction with both genes on the same vector. Plasmid pCMVdhfr·chLCHC is a co-linear plasmid with a murine dhfr encoding gene cassette.

Figure 6 shows flow cytometry data demonstrating binding of chimeric antibody conjugates to HUT78 cells. The conjugate is stained with a anti-human Fc specific antibody in panel A and anti-CLTB36 guinea pig serum in panel B.

Figure 7 illustrates anti-CLTB36 IgG titres in macaque sera as measured by ELISA, after immunization and boosting with ch·44H104-CLTB36 conjugates.

Figure 8 illustrates anti-rP24 IgG titres in bleed 1 and 4 of macaques immunized with ch. 44H104-CLTB36 conjugates.

Figure 9 depicts Coomassie blue stained SDS/PAGE gels 7.5% (A) and 10% (B). Gel A was run with samples in non-reducing buffer and gel B in reducing buffer. The bands corresponding to the intact antibody (A) and light and heavy chains (B) are labelled with arrows.

Figure 10 depicts Western blots corresponding to the Coomassie blue stained gels of Figure 9. The bands corresponding to intact antibody conjugate (A) and light and heavy chain conjugates (B) are indicated with arrows. The primary antibody reagent used was anti-CLTB36 guinea pig anti-sera.

GENERAL DESCRIPTION OF INVENTION

In the present invention, an antigen, against which it is desired to raise antibodies in a host, generally is conjugated to the C-terminus of both the light and heavy

chains of a monoclonal antibody, which is specific for a particular surface structure of antigen-presenting cells. This arrangement allows for delivery of the antigen to the relevant cells in the immune system upon injection of the conjugate to a host. The monoclonal antibody, therefore, acts as a "vector" or "delivery vehicle" for targeting antigenic determinants to antigen presenting cells, thereby facilitating their recognition by T-helper cells. The antigen presenting cells possess a variety of specific cell surface structures or markers which are targeted by any particular monoclonal antibody. Thus, antigens may be conjugated to a monoclonal antibody specific for any of the surface structures on the antigen presenting cells, including class I and class II major histocompatibility complex (MHC) gene products.

The surface structures on the antigen presenting cells of the immune system which can be recognized and targeted by the monoclonal antibody portion of the immunoconjugates are numerous and the specific surface antigen structure targeted by the monoclonal antibody depends on the specific monoclonal antibody.

The monoclonal antibody may be specific for a gene product of the MHC, and, in particular, may be specific for class I molecules of MHC or for class II molecules of MHC. However, the invention is not limited to such specific surface structures and the conjugates containing the corresponding monoclonal antibodies, but rather, as will be apparent to those skilled in the art, the invention is applicable to any other convenient surface structure of antigen presenting cells which can be recognized and targeted by a specific monoclonal antibody to which an immunogenic molecule is conjugated.

For example, strong adjuvant-independent serological responses to a delivered antigen can be obtained with conjugates formed with dendritic cell-specific monoclonal antibody and $CD4^+$ cell-specific monoclonal antibody.

In the present invention, the monoclonal antibody specific for the target structure is provided in the form of a conjugate with an antigen against which it is desired to elicit an immune response conveniently joined to the C-terminal of the heavy and/or light chains of the monoclonal antibody. While the conjugate antibody molecules are illustrated by such C-terminal connection, the antigen moiety alternatively may be inserted within the light and heavy chains of the antibody and such insertions may establish a particular constrained conformation of the antigen and, in particular, epitopes, within the known structural framework of an antibody molecule. Such conjugate antibody molecules may be conveniently produced by genetic modification of a gene encoding the heavy and light chains of the antibody to contain a gene encoding one or more antigen(s) and coexpressing the resulting nucleic acid molecules.

The invention is particularly useful for antigen molecules which normally possess a weakly-immunogenic response, since that the response is potentiated by the present invention. The antigen molecule may be in the form of a peptide or protein, as discussed above, but is not limited to such materials.

The present invention is applicable to any antigen which it is desired to target to antigen presenting cells using the monoclonal antibody. The antigen may be a protein or a peptide of 6 to 100 amino acids comprising an amino acid sequence of an epitope. Representative organisms from which the antigen may be derived include influenza viruses, parainfluenza viruses, respiratory viruses, measles viruses, mumps viruses, human immunodeficiency viruses, polio viruses, rubella viruses, herpex simplex viruses type 1 and 2, hepatitis viruses types A, B and C, yellow fever viruses, smallpox viruses, rabies viruses, vaccinia viruses, reo viruses, rhinoviruses, Coxsackie viruses, Echoviruses,

rotaviruses, papilloma viruses, paravoviruses and adenoviruses, *E. coli, V. cholera, BCG, M. tuberculosis, C. diphtheria, Y. pestis, S. typhi, B. pertussis, S. aureus, S. pneumoniae, S. pyogenes, S. mutans,* Myocoplasmas, Yeasts, *C. tetani,* meningococci (e.g., *N. meningitidis*), *Plasmodium* spp, *Mycobacteria* spp, *Shigella* spp, *Campylobacter* spp, *Proteus* spp, *Neisseria gonorrhoea*, and *Haemophilus influenzae*. The antigen moiety may also be derived from hormones, such as human HCG hormone, and tumor-associated antigens.

The present invention attempts to address some of the problems of the prior art, referred to above, by incorporating a peptide antigen, at the C-terminus of light and heavy chains of the targeting antibody by recombinant DNA means. The model peptide used herein is CLTB36, which is a tandem T-B HIV peptide found to elicit neutralizing responses in several animals (as described in copending USSN 08/257,528 filed June 9, 1994, assigned to the assignee hereof and the disclosure of which is incorporated herein by reference), although the principles of the invention are applicable to any antigen. The DNA sequence encoding this peptide is incorporated at the 3′ ends of the genes encoding a mouse/human chimeric anti-human class II mab (44H104), When these genes are included in a suitable expression vector and expressed, a recombinant chimeric anti-human class II/antigen fusion is obtained. This may be purified easily in a single step by Protein A affinity purification or other suitable procedure.

The present disclosure reports the *in-vivo* responses of macaques to a priming and boosting dose of anti-class II chimeric antibody/CLTB36 fusion generated by recombinant means. The genes for the fusion protein were generated by polymerase chain reaction (PCR) using cloned cDNA and synthetic oligonucleotides. The antigen

(CLTB36) gene was constructed using overlap extension PCR. The genes were cloned into an expression vector, transfected into YB2/0 cells and gene amplification carried out using a murine dhfr cassette cloned into the same expression plasmid. Several clones secreting adequate levels of the properly folded and assembled product were identified. The antigen fusions at the C-terminus of the light and heavy chain do not affect the proper assembly of the antibody (see Figure 9) which also maintains its binding specificity (see Figure 6).

As described in U.S. Patents Nos. 4,950,480 and 5,194,254, coupling a weak antigen to the specific monoclonal antibody results in an enhancement of the immunogenicity of such antigen, while avoiding the use of adjuvants and hence represents a much safer immunization procedure which can utilize materials from which only a weak immune response is achieved. Examples of such materials are small peptides which are epitopes of larger proteins or are protein subunits of a pathogen.

For human use, it is desirable that the antibody be modified to produce a mouse/human chimeric antibody, since extensive anti-murine monoclonal antibody responses would be generated by administration of a murine antibody to humans. Since the invention is broadly applicable to any species, it is desirable that, when a conjugate antibody molecule is administered to a specific species, the murine antibody sequences be replaced by corresponding sequences from the specific species in an analogous manner to that described herein for the mouse/human chimeric antibodies.

The experimental data presented herein and detailed in the Examples below demonstrate the ability of a mouse/human chimeric antibody, which targets antigen presenting cells (APC's) of the immune system via their surface MHC class II receptors, to enhance the immune response to a peptide antigen conjugated to the C

terminus of both the light and heavy chains. Such a conjugate can be produced conveniently, as detailed in the Examples, using recombinant DNA methodology, namely by assembling the genes encoding both the light and heavy chains with CLTB36 or other antigen of interest in a suitable expression vector. The vector pRC/CMV was selected as the basic expression plasmid in the experimental work performed herein, since it uses the powerful and broad host range immediate early CMV promoter to drive transcription. The final construct was designed to contain both light and heavy chain genes on the same vector as independent transcriptional units. The murine dhfr gene encoding cassette was also incorporated in this specific vector to provide a suitable means of gene amplification. This expression vector was electroporated into rat myeloma YB2/0 cells. Cell lines expressing recombinant antibody were established. Using the amplification procedure outlined in the Examples below and reported in the literature (ref. 32) stable cell lines secreting viable amounts of recombinant antibody conjugate (approximately 30 μg/ml) established relatively quickly (in about 4 months). The recombinant chimeric conjugate is assembled correctly and has the same specificity as the parent mab 44H104.

The recombinant conjugate, when administered to macaques without an extrinsic adjuvant (e.g. alum or syntex), elicits good priming immune response, as measured by IgG titres to the peptide antigen on the conjugate. This response is also directed towards the native antigen as measured by recombinant P24 reactivity. The priming response fades after a while but was boosted in two out of three animals by another dose of the chimeric mab conjugate in PBS.

The experimental data presented herein and detailed below, demonstrates the enhancement of immune response to a peptide antigen in the absence of conventional

adjuvants, by coupling to an anti-class II chimeric antibody, the conjugate being generated by recombinant means. The conjugate can be obtained in large amounts by expression in cells, such as YB2/O cells.

It is clearly apparent to one skilled in the art, that the various embodiments of the present invention have many applications in the fields of vaccination, diagnosis and treatment of diseases produced by selected pathogens. A further non-limiting discussion of such uses is further presented below.

1. Vaccine Preparation and Use

Immunogenic compositions, suitable to be used as vaccines, may be prepared from the conjugate antibody molecules as disclosed herein. The vaccine elicits an immune response in a subject which produces antibodies including anti-antigen moiety antibodies. Should the vaccinated subject be challenged by a pathogen that produces the antigen moiety, the antibodies bind to and inactivate the pathogen.

Immunogenic compositions including vaccines may be prepared as injectables, as liquid solutions or emulsions. The conjugate antibody molecules may be mixed with pharmaceutically acceptable excipients which are compatible therewith. Such excipients may include, water, saline, dextrose, glycerol, ethanol, and combinations thereof. The immunogenic compositions and vaccines may further contain auxiliary substances, such as wetting or emulsifying agents, or pH buffering agents. Immunogenic compositions and vaccines may be administered parenterally, by injection subcutaneously or intramuscularly. Alternatively, the immunogenic compositions formed according to the present invention, may be formulated and delivered in a manner to evoke an immune response at mucosal surfaces. Thus, the immunogenic composition may be administered to mucosal surfaces by, for example, the nasal or oral

(intragastric) routes. Alternatively, other modes of administration including suppositories and oral formulations may be desirable. For suppositories, binders and carriers may include, for example, polyalkalene glycols or triglycerides. Oral formulations may include normally employed incipients such as, for example, pharmaceutical grades of saccharine, cellulose and magnesium carbonate. These compositions can take the form of solutions, suspensions, tablets, pills, capsules, sustained release formulations or powders and contain about 1 to 95% of the conjugate antibody molecules. The immunogenic preparations and vaccines are administered in a manner compatible with the dosage formulation, and in such amount as will be therapeutically effective, protective and immunogenic. The quantity to be administered depends on the subject to be treated, including, for example, the capacity of the individual's immune-system to synthesize antibodies, and if needed, to produce a cell-mediated immune response. Precise amounts of active ingredient required to be administered depend on the judgment of the practitioner. However, suitable dosage ranges are readily determinable by one skilled in the art and may be of the order of micrograms or milligrams of the conjugate antibody molecules. Suitable regimes for initial administration and booster doses are also variable, but may include an initial administration followed by subsequent administrations. The dosage may also depend on the route of administration and will vary according to the size of the host.

The concentration of antigen in an immunogenic composition according to the invention is in general about 1 to 95%. A vaccine which contains antigenic material of only one pathogen is a monovalent vaccine. Vaccines which contain antigenic material of several pathogens are combined vaccines and also belong to the present invention. Such combined vaccines contain, for

example, material from various pathogens or from various strains of the same pathogen, or from combinations of various pathogens.

The nucleic acid molecules encoding the conjugate antibody molecules of the present invention may also be used directly for immunization by administration of the DNA directly, for example, by injection for genetic immunization. Processes for the direct injection of DNA into test subjects for genetic immunization are described in, for example, Ulmer et al, 1993 (ref. 33).

2. Immunoassays

The conjugate antibody molecules of the present invention are useful as immunogens for the generation of anti-antigen moiety antibodies (including monoclonal antibodies for use in immunoassays including enzyme - linked immunosorbent assays (ELISA), RIAs and other non-enzyme linked antibody binding assays or procedures known in the art. In ELISA assays, the anti-antigen moiety antibodies are immobilized onto a selected surface, for example, a surface capable of binding proteins such as the wells of a polystyrene microtiter plate. After washing to remove incompletely adsorbed antibodies, a nonspecific protein such as a solution of bovine serum albumin (BSA) that is known to be antigenically neutral with regard to the test sample may be bound to the selected surface. This allows for blocking of nonspecific adsorption sites on the immobilizing surface and thus reduces the background caused by nonspecific bindings of test sample onto the surface.

The immobilizing surface is then contacted with a sample, such as clinical or biological materials, to be tested in a manner conducive to immune complex (antigen/antibody) formation. This may include diluting the sample with diluents, such as solutions of BSA, bovine gamma globulin (BGG) and/or phosphate buffered saline (PBS)/Tween. The sample is then allowed to

incubate for from 2 to 4 hours, at temperatures such as of the order of about 25° to 37°C. Following incubation, the sample-contacted surface is washed to remove non-immunocomplexed material. The washing procedure may include washing with a solution, such as PBS/Tween or a borate buffer. Following formation of specific immunocomplexes between the test sample and the bound anti-antigenic moiety antibodies, and subsequent washing, the occurrence, and even amount, of immunocomplex formation may be determined.

Biological Deposits

Plasmid pCMVdhfr.chLCHC that contains portions coding for conjugate antibody molecules that is described and referred to herein has been deposited with the American Type Culture Collection (ATCC) located at 12301 Parklawn Drive, Rockville, Maryland, USA, 20852, pursuant to the Budapest Treaty and prior to the filing of this application, under Accession No. on . Samples of the deposited plasmid will become available to the public upon grant of a patent based upon this United States patent application. The invention described and claimed herein is not to be limited in scope by plasmid deposited, since the deposited embodiment is intended only as an illustration of the invention. Any equivalent or similar plasmids that encode similar or equivalent antigens as described in this application are within the scope of the invention.

EXAMPLES

The above disclosure generally describes the present invention. A more complete understanding can be obtained by reference to the following specific Examples. These Examples are described solely for purposes of illustration and are not intended to limit the scope of the invention. Changes in form and substitution of equivalents are contemplated as circumstances may suggest or render expedient. Although specific terms have been

employed herein, such terms are intended in a descriptive sense and not for purposes of limitations.

Enzymes and reagents commonly used in standard recombinant DNA technology manipulations were purchased from Böehringer Mannheim, New England Biolabs, Gibco/BRL and Pharmacia. Many specific reactions were performed using Reagent Kits which were purchased from several sources indicated in the specific Examples below. Antibody reagents for ELISAs were purchased from Caltag unless otherwise indicated. Plasmid vectors were purchased from Gibco/BRL or Invitrogen. Polymerase Chain Reaction (PCR) was performed using protocols and kits (Gene Amp PCR System) supplied by Perkin Elmer Cetus. The Thermal cycler used in PCR reactions was purchased from Perkin Elmer Cetus.

The synthesis of oligonucleotides was carried out using an Applied Biosystems 380B DNA synthesizer. The synthesized oligonucleotides were purified on OPC cartridges supplied by Applied Biosystems following the manufacturers protocols. DNA sequencing was performed on an automated DNA sequencer (370A; Applied Biosystems), using the dideoxy terminator chemistry and reagents supplied by the manufacturer.

Example 1:

This Example illustrates cDNA synthesis and sequence determination.

The hybridoma cell line 44H104 secreting murine anti-human class II mab (IgG2aK) was grown in RPMI medium, (Gibco-BRL) supplemented with glutamine (2mM), penicillin (50ug/ml) and streptomycin (50U/ml) and containing 10% FBS. Cells (10^6) were harvested and mRNA isolated using a 'Fast Track mRNA Isolation' kit (Invitrogen). First and second-strand cDNA was prepared using the 'cDNA synthesis Plus' kit (Amersham) and protocols supplied by the manufacturer. The cDNA generated in this step was cloned into λgt10 using the

'cDNA Cloning System-λgt10' kit (Amersham) to generate a lamda phage cDNA library. A cDNA library from the mRNA of mab 44H104 secreting cell line was made in lambda phage. Phage clones containing genes encoding the light and heavy chains were identified. PCR reactions were also performed on the cDNA (50 ng) using primers and conditions used by Winter and colleagues (Ref 28). The amplified products corresponding to V_L and V_H of 44H104 were labelled with P^{32} using the 'Random priming system I' kit (New England Biolabs) and used as probes to isolate phage clones containing inserts encoding the light and heavy chain genes.

The inserts were excised and cloned into the multilinker region of pUC18. These were sequenced and the nucleotide sequence of both V_L and V_H are displayed in Figure 1 and 1B respectively (SEQ ID Nos: 1 and 2). The italicised sequences in this figure are the sequences of the signal peptide which precede the mature sequences of the light and heavy chains. Most standard manipulations were performed using well described protocols (ref. 29).

Example 2:

This Example illustrates construction of a gene encoding peptide antigen CTLB36.

Antigen peptide CLTB36 (Figure 2A, SEQ ID No: 5), which consists of a tandemly linked T and B cell epitope, derived from the sequence of MN strain of HIV, was constructed by PCR using the overlap extension method (illustrated in Figure 2B). The nucleic acid sequence encoding CLTB36 was deduced from the amino acid sequence of the peptide antigen (Figure 2A, SEQ ID No: 6). The procedure consists of synthesizing three oligonucleotides (CLTB36.1, CLTB36.2 and CLTB36.3; Figure 2C, SEQ ID Nos: 7, 8 and 9) which span the entire gene. The oligonucleotide CLTB36.1 was designed to have 16 bases at the 3' end, complementary (overlap) to the 5' end of CLTB36.2, which in turn has a 16 base overlap at its 3'

end with corresponding 5′ nucleotides of oligonucleotide CLTB36.3. Polynucleotide primers designated as PrLC.F and PrHC.F were also synthesized; these were designed to overlap with the 5′ of the gene coding for CLTB36 and provide a *Bam*HI site for incorporation into the light chain gene or a *Kpn* I site for fusion with the heavy chain gene (Figure 2C, SEQ ID Nos: 10 and 11). The last primer(Pr.R) is the 'back' primer and has homology to the 3′ end of the CLTB36 gene and was designed to provide a *Hind* III site for cloning into the expression plasmid (Figure 2C, SEQ ID No: 12).

The oligonucleotides CLTB36.1, CLTB36.2, and CLTB36.3 were mixed together (30 pm each) in PCR reaction buffer heated up to 90°C and slowly annealed at about 45°C. Subsequently the volume was made up to 100 μl by adequate additions of buffer, dNTP's primers (PrLC.F and PrR for light chain antigen; PrHC.F and Pr.R for heavy chain antigen; 100 pmol each) using material and protocols from a Gene Amp PCR kit and a PCR reaction was performed. The aqueous phase of the reaction mixture was removed to another tube and an aliquot (5 μl) was ligated into pCRII vector and cloned using a 'TA cloning kit' (Invitrogen). The insert was sequenced and clones containing the correct sequence excisable by the correct combination of restriction sites were established.

Example 3:

This Example illustrates assembly of the gene encoding the chimeric light chain of 44H104 conjugated to mab CTLB36.

The V_L of 44H104 and its natural signal sequence was obtained by PCR amplification using pUC18·LC (pUC18 vector containing a light chain encoding cDNA insert) as a template. The two primers used in the reaction (Pr 1 and 2; Figure 3B, SEQ ID Nos: 13, 14) were designed to (a) incorporate a *Hind* III restriction site followed by a Kozak consensus sequence (CCGCC; ref. 3) at the 5′ of

the amplified product and (b) incorporate an *Xho* I restriction site at the junction of V_L and C_L by creating a silent mutation. The PCR reactions were carried out using 50 ng of template, 100 pmol each of the primers in a 100 μl volume using buffers, dNTP's and enzyme supplied in the GeneAmp kit. The cycling parameters were: 95°C for 1 min., 55°C for 1 min. followed by 72°C for 2 min., for a total of 25 cycles. An aqueous aliquot of the final reaction mixture was analyzed on a 10% agarose gel and another aliquot (5 μl) was ligated into pCRII vector supplied in the 'TA Cloning' kit (Invitrogen). The ligation reaction was used to transform competent *E. coli* cells plated out on X-Gal agar plates containing ampicillin. Plasmid was isolated from several colonies bearing a white phenotype and sequenced. Approximately one in three clones were found to have the correct sequence.

The human light chain constant (Kappa) gene required for the construct encoding chimeric 44H104 light chain was also obtained by PCR amplification. The template was a plasmid pUC19-k containing an insert coding for the human kappa gene. The primers used in the PCR reaction (pr. 3 and 4; Figure 3B, SEQ ID Nos: 15 and 16) were designed to incorporate an *Xho* I restriction site at the 5' end of the cassette suitable for ligation with the V_L gene obtained above. These primers also incorporate a *Bam*HI site at the 3' end to enable ligation to the antigen-CLTB36 gene. The PCR reaction was carried out in the same way as described above for V_L gene of 44H104, cloned into pCRII vector and clones carrying inserts identified and sequenced. Two clones having the correct sequence were set aside for further work.

The PCRII vector containing V_L gene insert was digested with a combination of *Hind* III and *Xho* I restriction endonucleases and the 400 bp insert isolated. Similarly polynucleotide fragments encoding the human

Kappa gene and CLTB36 were excised out of pCRII cloning vectors using digestion with combinations of *Xho* I/*BamH* I and *BamH* I/*Hind* III respectively. All three fragments were mixed (10-20 ng each) and ligated into an aliquot of *Hind* III digested expression plasmid pRC/CMV (Invitrogen) using standard protocols. The ligation reaction was used to transform competent *E. coli* TG1 cells and recombinants analyzed for inserts. The orientation of the insert was ascertained by restriction enzyme digest patterns and confirmed by DNA sequencing. This plasmid was designated as pCMV.chLC (Figure 5).

Example 4:

This Example illustrates assembly of a gene encoding the chimeric heavy chain of 44H104 mab conjugated with CTLB36.

The gene for the chimeric heavy chain conjugated to CLTB36 was constructed from gene cassettes, generated in a manner similar to what has been described for the light chain in Example 3. The detailed scheme and sequences of the oligonucleotide primers are shown in Figure 4. Synthetic oligonucleotide primers 5 and 6 (SEQ ID Nos: 17, 18) were used in generating the V_H gene from a plasmid template (pUC18) containing a cDNA insert encoding the heavy chain of mab 44H104. The primers were designed to incorporate a 5′ *Hind* III restriction site, a kozak sequence and a silent mutation at the 3′ (V_H-C_H junction) resulting in a *Spe* I site for ligation to the constant domain gene. The PCR product was cloned into pCRII vector and the nucleotide sequence integrity of the insert confirmed. The human constant domain (C γ1) gene was obtained by the amplification of the insert encoding this in plasmid pUC19-G1 using PCR primers 7 and 8 (SEQ ID Nos: 19, 20). As with primers Pr. 5 and Pr. 6, the primers were designed to engineer a 5′ *Spe* I site for ligation to the V_H gene and a *Kpn* I recognition site fusion to the antigen gene. The PCR products were cloned

into pCRII as before, and correct clones identified by DNA sequencing.

The gene cassettes encoding V_H, human Cγ1 and CLTB36 were obtained from sequences inserted into pRCII plasmid by digestion with combinations of *Hind* III/*Spe* I, *Spe* I/*Kpn* I and *Kpn* I/*Hind* III restriction enzymes respectively. The correct DNA fragments were isolated from agarose gels, mixed and ligated into *Hind* III digested pRC/CMV plasmid. These were used to transform competent *E.coli* cells and plasmid isolated from selected colonies. The plasmid was checked for inserts encoding the chimeric heavy chain-CLTB36 conjugate. The orientation of the gene with respect to the rest of the expression plasmid was established using restriction enzyme digestion patterns. The insert was also sequenced, the expression plasmid was designated pCMV.chHC (Figure 5).

Example 5

This Example illustrates construction of expression plasmids.

The DNA sequences encoding the CLTB36 fusions with chimeric light and heavy chains were assembled in pRC/CMV (Invitrogen) to give plasmids pCMV.chLC and pCMV.chHC respectively (Figure 5), as described in Examples 3 and 4. A single expression vector containing the genes for both light and heavy chains as distinct transcription units each under their own CMV promoter was constructed (the scheme is shown in Figure 5). pCMV.chHC plasmid was digested with *Nru* I and *Dra* III and a 2.8 kb DNA fragment isolated on a 0.8 % agarose gel. The DNA fragment was blunt ended following a standard protocol (ref. 30) and using dNTP's and DNA polymerase (Klenow). The resulting DNA fragment was then ligated into plasmid pCMV.chLC linearized by digestion with *Nru* I restriction enzyme and the resulting co-linear vector designated as pCMV.chLCHC. The orientation and general structure of the plasmid is

as shown in Figure 5 and was confirmed by extensive restriction enzyme digestion analysis.

Expression plasmid pCMVdhfr.chLCHC was constructed by inserting a blunt ended 1.9 kb *Pvu* II/*BamH* I fragment from plasmid pSV2.dhfr (ref. 31), into the *Bgl* II restriction site of vector pCMV.chLCHC. This DNA fragment encodes a murine dihydrofolate reductase gene under the control of a SV40 promoter and terminating in a SV40 poly A. The orientation of the insert was confirmed by restriction digest analysis and is as shown for pCMVdhfr.chLCHC in Figure 5. This plasmid was isolated from transformed TG1 cells by banding on cesium chloride (ref. 30) and used in transfection experiments.

Example 6:

This Example illustrates the expression of chimeric 44H104-CLTB36 conjugates.

Initial expression was attempted by co-transfecting plasmids pCMV. chLC and pCMV.chHC prepared as described in Examples 3 and 4, into non-Ig secreting murine SP2/0 myeloma cells by electroporation. The SP2/0 cells were grown to mid log phase and then harvested; $1X10^7$ cells were washed with cold PBS, centrifuged (4-5xg, for 5 min) and resuspended in 0.5 ml of PBS. Plasmid DNA linearized with *Bgl* II enzyme (10 μg of each plasmid) was added to the cell suspension and the mixture incubated on ice for 10 minutes. The suspension was transferred to a cold 0.4 cm electroporation cuvette and subjected to an electrical pulse at a setting of 700V and capacitance of 25 μF in a 'Gene Pulsar' electroporator (Biorad). The mixture was further incubated in ice (5 min.) and then left in supplemented RPMI (with 10% FBS) for 48 hours. Subsequently the cells were plated out in selective media consisting of RPMI medium supplemented with 10% FBS and 600 μg/ml of G418 (Sigma) in 96 well plates (1×10^4 cells per well). The media was replaced every three days and after 2 weeks, wells displaying cell growth were

checked for recombinant antibody secretion in supernatants by ELISA. Several pools/wells were selected and cloned by dilution cloning method (ref. 30) and again checked for ch. mab secretion. A few selected clones were expanded and stored as stocks with DMSO in liquid nitrogen. The expression plasmid pCMV.chLCHC was also used to transfect SP2/0 cells. The methodology of electroporation and establishment of cloned cell lines secreting chimeric mab-CLTB36 conjugates are as described above. The overall yield was, however, quite low.

The expression plasmid pCMVdhfr.chLCHC, prepared as described in Example 5, was transfected into YB2/0 rat myeloma cells (ATCC CRL 1662) following the protocols detailed by Shitara et al. (ref. 32). Essentially YB2/0 cells were grown in supplemented RPMI (containing 2mM glutamine; penicillin 50ug/ml and streptomycin 50 U/ml) containing 10% FBS. Aliquots of 1 x 10^7 cells were collected, washed in PBS and taken up in 250 μl of PBS. These were mixed with non-linearized plasmid pCMVdhfr.chLCHC (10 μg) and electroporated at 200V, 250 μF capacitance in a Gene Pulsar electroporator (Bio Rad). The cells were then treated exactly like the electroporated SP2/0 cells described above and after 48 hours in non selective media were plated into ten 96-well plates in supplemented RPMI containing 600 μg/ml of G418. The media from wells displaying cell growth were analyzed for recombinant antibody and pools secreting the desired product identified. Some selected pools were transferred to 6 well plates and the media was replaced with supplemented RPMI containing 10% FBS, 600 μg/ml G418 and 50 nM of methotrexate (Sigma). The pools were adapted to this concentration of methotrexate (MTX) and then the level was increased to 100 nM. Subsequently the concentration of MTX was increased to 200 nM, then 500 nM, 1000 nM and finally 1500 nM. The cells were adapted to each of these levels through several passages and

finally cloned by limiting dilution. Several clones secreting recombinant products from 3 to 30 μg/ml of spent culture medium (after protein A purification) were obtained and were used to obtain the chimeric mab in quantities large enough to permit experimentation in animals.

96 well microtitre plates (Maxisorp Immuno; Nunc) were coated with a Goat anti-human-kappa light chain antibody fragment. The plates were washed in PBST (PBS containing 0.05% Tween 20), blocked with 0.1% casein in PBST, and incubated with aliquots (100 μl) of culture supernatants. A human myeloma IgG1K (Pharmingen) was used as a positive control. After washing, the plates were incubated with a goat anti-human IgG (Fc specific) $F(ab')_2$ conjugated to alkaline phosphatase. The un-bound conjugate was washed out and substrate pNPP (Gibco/BRL) was added to the wells in phosphotase buffer. After about 15 min and the colour development measured in a Dynatech MR5000 ELISA plate reader at a setting of 405-410 nm.

Example 7:

This Example describes the isolation and purification of ch 44H104-CLTB36 conjugates.

Clones identified as high producers of conjugate in Example 6, exclusively from the pCMVdhfr.chLCHC transfection of YB2/0 cells and subsequent gene amplification experiments, were scaled up in supplemented RPMI containing G418 (600 μg/ml), methotrexate (1μM) and 10% ultra low IgG FBS (from Gibco/BRL). The cells were allowed to grow in T-flasks until approximately half of them were dead (approximately 1 week). The culture was centrifuged and the supernatant collected. The spent media was stored at 4°C with 0.1% sodium azide to prevent microbial growth.

The ch 44H104-CLTB36 conjugates in the supernatant were isolated by Protein A purification. The supernatant was passed through a Protein A-HyperD column (Sepracor).

The column was washed and the bound material eluted with 0.2M glycine (pH 2.8); the fractions containing bound material were neutralized in 1.0M Tris (pH 8.0) and pooled. The fractions were dialyzed against PBS and finally concentrated on Amicon micro-concentrators. The protein content of the pooled, dialyzed and concentrated material was determined using a Standard Protein Assay Kit (Biorad Laboratories). The conjugate was stored at 4°C in PBS.

To remove any high molecular weight aggregates, the Protein A purified material was further fractionated on a Sephacryl S-300 (HR; 9.5 x 90 cm) hplc column. The column was equilibrated with PBS and the sample applied in 2ml aliquots. The column was run at a flow rate of 1 ml/min in PBS and the effluent monitored at 280 nm. The void volume peak (consisting of any aggregates) was collected separately from the peak corresponding to the non-aggregated material. The latter fractions were pooled and concentrated using a YM-10 ultra filtration membrane (Amicon).

Example 8:

This Example describes characterization of ch mab 44H104-CLTB36 conjugate.

The conjugate produced following the procedure of Example 7 was assembled as a covalently linked dimer of heterodimers comprised of light and heavy chains. This was demonstrated by SDS/PAGE electrophoresis on 7.5 and 10% gels, running samples in non-reducing and reducing buffer respectively (see Figure 9). The presence of CLTB36 peptide on the conjugates was determined by Western blotting using anti-CLTB36 guinea pig serum generated in house. The second antibody used in these experiments was a Goat anti-guinea pig IgG-alkaline phosphatase conjugate (Jackson Laboratories) (see Figure 10).

The conjugate was also analyzed for binding to class

II molecules on HUT78 cells by Flow Cytometry using binding of recombinant conjugate to HUT78 cells. HUT78 cells (Human MHC class II expressing T cell lymphoma cells) were grown in supplemented RPMI containing 10% FBS. An aliquot of cells (1 x 10^6 cells/tube) was distributed into 15 ml conical centrifuge tubes and washed with 2 ml of binding buffer (PBS containing 0.1% BSA and 0.1% NaN_3). The cells were collected after centrifugation (400 x g for 5 min at 4°C) and the pellet resuspended in binding buffer containing different concentrations of recombinant antibody conjugate (Figure 8). The tubes were incubated on ice for 60 minutes with occasional shaking and then washed twice with chilled (4°C) washing buffer (2 ml). The cells were suspended in 100 µl of a 1:20 dilution of fluoroscein isothiocyanate-conjugated goat anti-human IgG (Fc specific; Sigma Chemical Co.) and incubated further on ice for 30 minutes with occasional agitation. The cells were washed in binding buffer(2X) and subsequently once in PBS containing 0.1% sodium azide (NaN_3). The cells were finally suspended in an aliquot of 1% paraformaldehyde in PBS (0.5 ml) and analyzed in the EPIC V flowcytometer (Coulter, Harpendon UK).

The recombinant conjugate was also analyzed for the presence of CLTB36 peptide by the same technique. For this analysis ,the anti-human conjugate in the above protocol was substituted with anti-CLTB36 guinea pig serum generated in house. This step was followed by 100 µl of 1:50 dilution of biotin-conjugated mouse IgG2b anti-guinea pig mab (sigma) for 30 minutes and finally with 100 µl of a 1:5 dilution of a streptavidin-phycoerythrin conjugate (Becton Dickinson; 30 min). Cells were washed as before and fixed with 1% paraformaldehyde in PBS and analyzed in the flowcytometer. Negative controls, consisting of cells treated as described above but without the incubation

step with recombinant mab conjugate, were used in both assays.

The results obtained are shown in Figure 6. This analysis demonstrates the availability on the surface of cells of the peptide for binding to antibody.

Example 9:

This Example describes immunization of macaques with ch 44H104-CLTB36 conjugates.

The immunogen (mab conjugate), prepared as in Example 7, was concentrated and filtered through a 0.22 μM filter. The protein concentration of this was estimated to be about 0.58 mg/ml in PBS.

Three cynomologous macaques were selected and serum samples from them these were screened for adventitious viral agents, such as SA8, HSV-1, HSV-2, V. Zoster, Chimp CMV, EBV, SRV-1, SRV-2, SRV-5, SIV, STLV-1, and B virus. The selected macaques (#197, 198 and 200) were bled and injected intramuscularly with 1.5 ml of PBS (containing 800 μg of protein, equivalent to 80 μg of peptide). The schedule set forth in the following Table 1 was established.

TABLE 1

Week	Procedure
0	Pre-bleed Primary injection (0.8 mg of conjugate each)
2	Bleed 1
4	Bleed 2
6	Bleed 3 Boost 1 (0.8 μg of conjugate each)
8	Bleed 4
10	Bleed 5

The serum samples from the pre-bleed and Bleeds 1 to 5 were screened for anti-CLTB36 reactivity.

96 well microtitre plates (Polystyrene; Dynatech Labs) were coated with 10 μg/ml of CLTB36 in Carbonate-Bicarbonate buffer (0.05M; pH 9.6). The wells were blocked with 5% skim milk in PBS and subsequently washed in PBS-Tween 20 (0.05%). The serum samples were diluted serially (in 1% skim milk with 0.05% Tween 20) into the wells and incubated at 37°C for 2 hours. The plates were washed and incubated with Goat anti-monkey IgG $F(ab')_2$ conjugated to Horse Radish Peroxidase (Cappel Laboratories). The excess conjugate was washed off and the colorimetric substrate TMB/H_2O_2 (ADI) added. The reaction was stopped after 5 min and absorbance measured at 450 and 540 nm in an ELISA Plate reader (EL 310; Biotech Instruments).

The protocol and reagents for an ELISA for P24 reactivity were as described for CLTB36 above; the difference being that the 96 well microtitre plates were coated with recombinant P24 (Dupont) at 1 μg/ml concentration in Carbonate-Bicarbonate buffer.

The IgG titres in different bleeds reactive against CLTB36 and measured by ELISA, are shown in Figure 7. As may be seen, good priming responses were elicited by the recombinant targeting conjugate in PBS, in all three animals (up to about 1 in 25,000 in one animal). The observed ELISA titres diminish after 4 and 6 weeks and then increase again after a boosting dose of the immunogen. The boost in IgG titres was especially prominent in two animals out of the three, the third for unexplained reasons did not boost after such a promising primary response.

The pre-bleed monkey sera and Bleed 1 and 4 (2 weeks post priming and 2 weeks post boosting respectively) were also evaluated for IgG responses against recombinant P24 (CLTB36 has an epitope derived from this portion of HIV

protein). Detectable P24 titres were measured in all three animals and are presented in Figure 8.

SUMMARY OF DISCLOSURE

In summary of this disclosure, the present invention provides novel recombinantly-produced molecules containing an antigen moiety and a monoclonal antibody moiety, wherein the monoclonal antibody moiety is specific for a determinant expressed on antigen-presenting cells of a host, procedures for assembly of such molecules, nucleic acid molecules encoding such molecules and immunizing procedures using such molecules, whereby an enhanced immune response to the antigen moiety is achieved in the absence of adjuvants. Modifications are possible within the scope of this invention.

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CLAIMS

What we claim is:

1. A recombinant conjugate antibody molecule, comprising a monoclonal antibody moiety specific for a surface structure of antigen presenting cells genetically modified to contain at least one antigen moiety exclusively at at least one preselected site on said monoclonal antibody moiety, whereby said conjugate antibody molecule is capable of delivering said antigen moiety to the antigen presenting cells of a host and capable of eliciting an immune response to said antigen moiety in the host.

2. The molecule of claim 1 wherein said antigen presenting cells are selected from the group consisting of class I major histocompatibility expressing cells, class II major histocompatibility expressing cells, dendritic cells and $CD4^+$ cells.

3. The molecule of claim 1 wherein said at least one antigen moiety is located at at least one end of at least one of the heavy and light chains of said monoclonal antibody moiety.

4. The molecule of claim 3 wherein said at least one antigen moiety is located at the C-terminal end of said at least one of the heavy and light chains of said monoclonal antibody moiety.

5. The molecule of claim 4 wherein said at least one antigen moiety is located at the C-terminal end of both said heavy and light chains of said monoclonal antibody moiety.

6. The molecule of claim 5 wherein said at least one antigen moiety is directly linked to the C-terminal end of both said heavy and light chains of said monoclonal antibody moiety.

7. The molecule of claim 6 wherein said at least one antigen moiety is an inherently weakly-immunogenic antigen moiety.

8. The molecule of claim 6 wherein said at least one antigen moiety comprises a plurality of antigen moieties.

9. The molecule of claim 8 wherein said plurality of antigen moieties is a plurality of a single antigen moiety.

10. The molecule of claim 8 wherein said plurality of antigen moieties is a plurality of different antigenic moieties.

11. The molecule of claim 7 wherein said at least one antigen moiety is a peptide having from 6 to 100 amino acids and containing at least one epitope.

12. A nucleic acid molecule, comprising a first nucleotide sequence encoding a chain of a monoclonal antibody specific for a surface structure of antigen-presenting cells selected from the group consisting of the heavy chain and the light chain of the monoclonal antibody, a second nucleotide sequence encoding at least one antigen and a third nucleotide sequence comprising a promoter for eukaryotic cell expression of a fusion protein comprising said monoclonal antibody chain and said at least one antigen.

13. The nucleic acid molecule of claim 12 wherein said encoded chain is the heavy chain of the monoclonal antibody.

14. The nucleic acid molecule of claim 12 wherein said encoded chain is the light chain of the monoclonal antibody.

15. The nucleic acid molecule of claim 12 wherein antigen presenting cells are selected from the group consisting of class I major histocompatibility expressing cells, class II major histocompatibility expressing cells, dendritic cells and $CD4^+$ cells.

16. The nucleic acid molecule of claim 12 wherein said first nucleotide sequence and said second nucleotide sequence are directly linked in a single transcriptional unit under control of said promoter.

17. The nucleic acid molecule of claim 16 wherein said third nucleotide sequence is disposed at the 5′ end of said first nucleotide sequence.

18. A vector comprising the nucleic acid molecule of claim 12.

19. The vector of claim 18 containing a first nucleic acid molecule comprising a first nucleotide sequence encoding the heavy chain of a monoclonal antibody specific for a surface structure of antigen-presenting cells, a second nucleotide sequence encoding at least one antigen and a third nucleotide sequence comprising a promoter for eukaryotic cell expression of a fusion protein comprising said monoclonal antibody heavy chain and said at least one antigen as a first transcriptional unit, and a second nucleic acid molecule comprising a first nucleotide sequence encoding the light chain of a monoclonal antibody specific for a surface structure of antigen-presenting cells, a second nucleotide sequence encoding at least one antigen and a third nucleotide sequence comprising a promoter for eukaryotic cell expression of a fusion protein comprising said monoclonal antibody light chain and said at least one antigen as a second transcriptional unit.

20. The vector of claim 19 having the characteristic properties of plasmid pCMVdhfr.chLCHC.

21. A method of making a conjugate antibody molecule comprising a monoclonal antibody moiety specific for a surface structure of antigen-presenting cells and at least one antigen moiety, which comprises:

constructing a first nucleic acid molecule containing a first nucleotide sequence encoding a heavy chain of said monoclonal antibody and a second nucleotide sequence encoding at least one antigen,

constructing a second nucleotide acid molecule containing a first nucleotide sequence encoding a light chain of said monoclonal antibody and a second nucleotide

sequence encoding said at least one antigen, and

coexpressing said first and second nucleic acid molecules in mammalian cells to form said conjugate antibody molecule.

22. The method of claim 21 wherein said coexpression of said first and second nucleic acid molecules includes constructing an expression vector containing said first and second nucleic acid molecules as independent transcriptional units.

23. The method of claim 22 wherein each said independent transcriptional unit includes a promoter operable in mammalian cells to direct said coexpression.

24. The method of claim 23 wherein said expression vector has the characteristic properties of plasmid pCMVdhfr.chLCHC.

25. The method of claim 23 wherein said coexpression includes secretion of said conjugate antibody molecule and further separating and purifying said conjugate antibody molecule.

26. The method of claim 25 wherein said purification comprises binding of the conjugate antibody molecule to protein A and selective elution of said conjugate antibody molecule from protein A.

27. An immunogenic composition, comprising a conjugate antibody molecule comprising a monoclonal antibody moiety specific for a surface structure of antigen presenting cells genetically modified to contain at least one antigen moiety exclusively at at least one preselected site on said monoclonal antibody moiety, whereby said conjugate antibody molecule is capable of delivering said antigen moiety to the antigen presenting cells of a host and capable of eliciting an immune response to said antigen moiety in the host or a nucleic acid molecule comprising a first nucleotide sequence encoding a chain of a monoclonal antibody specific for a surface structure of antigen-presenting cells selected from the group

consisting of the heavy chain and the light chain of the monoclonal antibody, a second nucleotide sequence encoding at least one antigen and a third nucleotide sequence comprising a promoter for eukaryotic cell expression of a fusion protein comprising said monoclonal antibody chain and said at least one antigen.

28. The immunogenic composition of claim 27 formulated as a vaccine for *in vivo* administration to a host to confer protection against disease caused by a pathogen producing said at least one antigen.

29. A method of generating an immune response in a host, comprising administering thereto an immuno-effective amount of an immunogenic composition comprising a conjugate antibody molecule comprising a monoclonal antibody moiety specific for a surface structure of antigen presenting cells genetically modified to contain at least one antigen moiety exclusively at at least one preselected site on said monoclonal antibody moiety, whereby said conjugate antibody molecule is capable of delivering said antigen moiety to the antigen presenting cells of a host and capable of eliciting an immune response to said antigen moiety in the host or a nucleic acid molecule comprising a first nucleotide sequence encoding a chain of a monoclonal antibody specific for a surface structure of antigen-presenting cells selected from the group consisting of the heavy chain and the light chain of the monoclonal antibody, a second nucleotide sequence encoding at least one antigen and a third nucleotide sequence comprising a promoter for eukaryotic cell expression of a fusion protein comprising said monoclonal antibody chain and said at least one antigen.

30. A method of determining the presence of a selected antigen in a sample, which comprises:

(a) immunizing a host with a conjugate antibody molecule, comprising a monoclonal antibody moiety

specific for a surface structure of antigen presenting cells genetically modified to contain at least one antigen moiety exclusively at at least one preselected site on said monoclonal antibody moiety, whereby said conjugate antibody molecule is capable of delivering said antigen moiety to the antigen presenting cells of a host and capable of eliciting an immune response to said antigen moiety in the host, wherein said at least one antigen moiety is said selected antigen to produce antibodies specific to said selected antigen;

(b) isolating said antibodies;

(c) contacting the sample with the isolated antibodies to produce complexes comprising any selected antigen in the sample and said selected antigen-specific antibodies; and

(d) determining production of the complexes.

31. A diagnostic kit for determining the presence of a selected antigen in a sample, comprising:

(a) a conjugate antibody molecule comprising a monoclonal antibody moiety specific for a surface structure of antigen presenting cells genetically modified to contain at least one antigen moiety exclusively at at least one preselected site on said monoclonal antibody moiety, whereby said conjugate antibody molecule is capable of delivering said antigen moiety to the antigen presenting cells of a host and capable of eliciting an immune response to said antigen moiety in the host, wherein the at least one antigen moiety is said selected antigen;

(b) means for detecting the production of complexes comprising any selected antigen in the sample and selected antigen-specific antibodies to said selected antigen; and

(c) means for determining production of the complexes.

32. A method for producing antibodies specific for a

selected antigen comprising:

(a) immunizing a host with an effective amount of an immunogenic composition comprising a conjugate antibody molecule comprising a monoclonal antibody moiety specific for a surface structure of antigen presenting cells genetically modified to contain at least one antigen moiety exclusively at at least one preselected site on said monoclonal antibody moiety, whereby said conjugate antibody molecule is capable of delivering said antigen moiety to the antigen presenting cells of a host and capable of eliciting an immune response to said antigen moiety in the host or a nucleic acid molecule comprising a first nucleotide sequence encoding a chain of a monoclonal antibody specific for a surface structure of antigen-presenting cells selected from the group consisting of the heavy chain and the light chain of the monoclonal antibody, a second nucleotide sequence encoding at least one antigen and a third nucleotide sequence comprising a promoter for eukaryotic cell expression of a fusion protein comprising said monoclonal antibody chain and said at least one antigen, wherein said at least one antigen is the selected antigen to produce antibodies specific for the selected antigen; and

(b) isolating the antibodies from the host.

33. A method of producing monoclonal antibodies specific for a selected antigen comprising:

(a) administering an immunogenic composition comprising a conjugate molecule comprising a monoclonal antibody moiety specific for a surface structure of antigen presenting cells genetically modified to contain at least one antigen moiety exclusively at at least one preselected site on said monoclonal antibody moiety, whereby said conjugate antibody molecule is capable of delivering said antigen moiety to the antigen presenting cells of a host and capable of eliciting an immune response to

said antigen moiety in the host, or a nucleic acid molecule comprising a first nucleotide sequence encoding a chain of a monoclonal antibody specific for a surface structure of antigen-presenting cells selected from the group consisting of the heavy chain and the light chain of the monoclonal antibody, a second nucleotide sequence encoding at least one antigen and a third nucleotide sequence comprising a promoter for eukaryotic cell expression of a fusion protein comprising said monoclonal antibody chain and said at least one antigen, wherein said at least one antigen is the selected antigen to at least one mouse to produce at least one immunized mouse;

(b) removing B-lymphocytes from the at least one immunized mouse;

(c) fusing the B-lymphocytes from the at least one immunized mouse with myeloma cells, thereby producing hybridomas;

(d) cloning the hybridomas;

(e) selecting clones which produce anti-selected antigen antibody;

(f) culturing the anti-selected antigen antibody-producing clones; and then

(g) isolating anti-selected antigen antibodies from the cultures.

ABSTRACT OF THE DISCLOSURE

Antibody molecules specific for surface structures of antigen presenting cells that have been modified to include an antigen moiety at a specific site therein to produce novel conjugate antibody molecules are disclosed. These conjugate molecules are produced by genetic modification of genes encoding light and heavy chains of the surface structure specific antibody, and expression in mammalian cells to produce the conjugate antibody. The conjugate antibody retained specificity for antigen presenting cells and contained the antigen moiety. The conjugate antibody molecules deliver the antigen to antigen presenting cells to produce an enhanced immune response to a host immunized therewith.

The conjugate antibody molecules and nucleic acid molecules encoding them are useful as antigens and as immunogens in diagnostic and prophylactic applications.

<u>**Combined Declaration and Power of Attorney for United States Patent Application**</u>

As a below named inventor, I hereby declare that:

My residence, post office address and citizenship are as stated below next to my name;

I believe I am an original, first and joint inventor of the subject matter which is claimed and for which a patent is sought on the invention entitled: **CHIMERIC ANTIBODIES FOR DELIVERY OF ANTIGENS TO SELECTED CELLS OF THE IMMUNE SYSTEM**, the specification of which is attached hereto.

I hereby state that I have reviewed and understand the contents of the above identified specification, including the claims, as amended by any amendment referred to above.

I acknowledge the duty to disclose information which is material to the examination of this application in accordance with Title 37, Code of Federal Regulations, S.1.56(a).

I hereby claim foreign priority benefits under Title 35, United States Code, S.119 of any foreign application(s) for patent or inventor's certificate listed below and have also identified below any foreign application for patent or inventor's certificate having a filing date before that of the application on which priority is claimed:

Prior Foreign Application(s)			Priority Claimed Yes	Priority Claimed No
(Number)	(Country)	(Day/Month/Year Filed)		

I hereby claim the benefit under Title 35, United States Code, S.120 of any United States application(s) listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States application in the manner provided by the first paragraph of Title 35, United States Code, S.112, I acknowledge the duty to disclose material information as defined in Title 37, Code of Federal Regulations, S.1.56(a) which occurred between the filing date of the prior application and the national or PCT international filing date of this application:

(Appln. Serial No.)	(Filing Date)	(Status) (patented, pending, abandoned)

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that wilful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such wilful false statements may jeopardize the validity of the application or any patent issued thereon.

I hereby appoint the following attorney(s) and/or agent(s) to prosecute this application and transact all business in the Patent and Trademark Office connected therewith:

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(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 387 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

ATGGACATGA GGGTTCCTGC TCACGTTTTT GGCTTCTTGT TGCTCTGGTT TCCAGGTACC 60

AGATGTGACA TCCAGATGAC CCAGTCTCCA TCCTCCTTAT CTGCCTCTCT GGGACAAAGA 120

GTCAGTCTCA CTTGTCGGGC AAGTCAGGAA ATTAGTGGTT ACTTAACCTG GCTTCAGCAG 180

AAACCAGATG GAACTATTAA ACGCCTGGTC TACGCCGCGT CCACTTTAGA TTCTGGTGTC 240

CCAAAAAGGT TCAGTGGCAG TAGGTCTGGG TCAGATTATT CTCTCACCAT CAGCAGCCTT 300

GAGTCTGAAG ATTTTGCAGA CTATTACTGT CTACAATATA CTAATTATCC GCTCACGTTC 360

GGTGCTGGGA CCAAGCTGGA GCTGAAA 387

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 129 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

Met Asp Met Arg Val Pro Ala His Val Phe Gly Phe Leu Leu Leu Trp
1 5 10 15

Phe Pro Gly Thr Arg Cys Asp Ile Gln Met Thr Gln Ser Pro Ser Ser
20 25 30

Leu Ser Ala Ser Leu Gly Gln Arg Val Ser Leu Thr Cys Arg Ala Ser
35 40 45

Gln Glu Ile Ser Gly Tyr Leu Thr Trp Leu Gln Gln Lys Pro Asp Gly
50 55 60

Thr Ile Lys Arg Leu Val Tyr Ala Ala Ser Thr Leu Asp Ser Gly Val
65 70 75 80

Pro Lys Arg Phe Ser Gly Ser Arg Ser Gly Ser Asp Thr Ser Leu Thr
85 90 95

Ile Ser Ser Leu Glu Ser Glu Asp Phe Ala Asp Tyr Tyr Cys Leu Gln
100 105 110

Tyr Thr Asn Tyr Pro Leu Thr Phe Gly Ala Gly Thr Lys Leu Glu Leu
115 120 125

Lys

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 420 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

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ATGGCTCTCC TGGTACTGTT CCTCTCCCTG GCTGCATTTC CAAGCTGTGG TGTCCTGTCC      60

CAGGTGCAGC TGAAGGAGTC AGGACCTGGC CTGGTGGCGC CCTCACAGAG CCTGTCCATC     120

ACTTGCACTG TCTCTGGGTT TTCATTAACC AGCTATGGTG TACACTGGGT TCGCCAGCCT     180

CCAGGAAAGG GTCTGGAGTG GCTGGGAGTA ATATGGGCTG GTGGAAGCAT AAATTATAAT     240

TCGGCTCTCA TGTCCAGACT GAGCATCAGC AAAGACAACT TCAAGAGCCA AGTTTTCTTA     300

AAAATGAGCA GTCTGCAAAC TGATGACACA GCCATGTACT ACTGTGCCAG AGCCTATGGT     360

GACTACGTCC ACTATGCTAT GGACTACTGG GGTCAAGGAA CCTCAGTCAC CGCCTCCTCA     420
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(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 140 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

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Met Ala Leu Leu Val Leu Phe Leu Ser Leu Ala Ala Phe Pro Ser Cys
1               5                  10                  15

Gly Val Leu Ser Gln Val Gln Leu Lys Glu Ser Gly Pro Gly Leu Val
            20                  25                  30

Ala Pro Ser Gln Ser Leu Ser Ile Thr Cys Thr Val Ser Gly Phe Ser
        35                  40                  45

Leu Thr Ser Tyr Gly Val His Trp Val Arg Gln Pro Pro Gly Lys Gly
    50                  55                  60

Leu Glu Trp Leu Gly Val Ile Trp Ala Gly Gly Ser Ile Asn Tyr Asn
65                  70                  75                  80

Ser Ala Leu Met Ser Arg Leu Ser Ile Ser Lys Asp Asn Phe Lys Ser
                85                  90                  95
```

Gln Val Phe Leu Lys Met Ser Ser Leu Gln Thr Asp Asp Thr Ala Met
100 105 110

Tyr Tyr Cys Ala Arg Ala Tyr Gly Asp Tyr Val His Tyr Ala Met Asp
115 120 125

Tyr Trp Gly Gln Gly Thr Ser Val Thr Ala Ser Ser
130 135 140

(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 34 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

Gly Pro Lys Glu Pro Phe Arg Asp Tyr Val Asp Arg Phe Tyr Lys Asn
1 5 10 15

Lys Arg Lys Arg Ile His Ile Gly Pro Gly Arg Ala Phe Tyr Thr Thr
20 25 30

Lys Asn

(2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 108 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

GGTCCTAAAG AACCTTTTAG AGACTATGTT GATAGGTTTT ATAAGAATAA GAGGAAGAGG 60

ATACATATAG GGCCTGGTAG GGCTTTTTAT ACTACTAAGA ATTAATAA 108

(2) INFORMATION FOR SEQ ID NO:7:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 60 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

CATTATGGAT CCGGTCCTAA AGAACCTTTT AGAGACTATG TTGATAGGTT TTATAAGAAT 60

(2) INFORMATION FOR SEQ ID NO:8:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 51 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

GCCCTACCAG GCCCTATATG TATCCTCTTC CTCTTATTCT TATAAAACCT A 51

(2) INFORMATION FOR SEQ ID NO:9:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 51 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

AGGGCCTGGT AGGGCTTTTT ATACTACTAA GAATTAATAA AAGCTTTAGC G 51

(2) INFORMATION FOR SEQ ID NO:10:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

CATTATGGAT CCGGTCCTAA 20

(2) INFORMATION FOR SEQ ID NO:11:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 30 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

GTCAGGTACC GGTCCTAAAG AACCTTTTAG 30

(2) INFORMATION FOR SEQ ID NO:12:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

GGCTAAAGCT TTTATTAATT C 21

(2) INFORMATION FOR SEQ ID NO:13:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 38 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:

AGCCTAAGCT TCCGCCATGG ACATGAGGGT TCCTGCTC 38

(2) INFORMATION FOR SEQ ID NO:14:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 33 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:14:

CCGTTTCAGC TCGAGCTTGG TCCCAGCACC GAA 33

(2) INFORMATION FOR SEQ ID NO:15:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 40 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:15:

CCTACTCGAG CTGAAACGGA CTGTGGCTGC ACCATCTGTC 40

(2) INFORMATION FOR SEQ ID NO:16:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 44 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:16:

ATTAAAGCTT TTACTAGGAT CCACACTCTC CCCTGTTGAA GCTC 44

(2) INFORMATION FOR SEQ ID NO:17:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 36 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:17:

AGCTAAGCTT CCGCCATGGC TCTCCTGGTA CTGTTC 36

(2) INFORMATION FOR SEQ ID NO:18:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 29 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:18:

GCGCACTAGT TCCTTGACCC CAGTAGTCC 29

(2) INFORMATION FOR SEQ ID NO:19:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 52 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:19:

GCGCACTAGT GTCACCGCCT CCTCAGCCTC CACCAAGGGC CCATCGGTCT TC 52

(2) INFORMATION FOR SEQ ID NO:20:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 43 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:20:

ACGCAAGCTT TTACTAGGTA CCTTTACCCG GAGACAGGGA GAG 43

VL sequence of the light chain of murine 44H104 mab:

```
ATG GAC ATG AGG GTT CCT GCT CAC GTT TTT GGC TTC TTG TTG CTC TGG TTT
MET Asp MET Arg Val Pro Ala His Val Phe Gly Phe Leu Leu Leu Trp Phe

CCA GGT ACC AGA TGT GAC ATC CAG ATG ACC CAG TCT CCA TCC TCC TTA TCT
Pro Gly Thr Arg Cys Asp Ile Gln MET Thr Gln Ser Pro Ser Ser Leu Ser

GCC TCT CTG GGA CAA AGA GTC AGT CTC ACT TGT CGG GCA AGT CAG GAA ATT
Ala Ser Leu Gly Gln Arg Val Ser Leu Thr Cys Arg Ala Ser Gln Glu Ile

AGT GGT TAC TTA ACC TGG CTT CAG CAG AAA CCA GAT GGA ACT ATT AAA CGC
Ser Gly Tyr Leu Thr Trp Leu Gln Gln Lys Pro Asp Gly Thr Ile Lys Arg

CTG GTC TAC GCC GCG TCC ACT TTA GAT TCT GGT GTC CCA AAA AGG TTC AGT
Leu Val Tyr Ala Ala Ser Thr Leu Asp Ser Gly Val Pro Lys Arg Phe Ser

GGC AGT AGG TCT GGG TCA GAT TAT TCT CTC ACC ATC AGC AGC CTT GAG TCT
Gly Ser Arg Ser Gly Ser Asp Tyr Ser Leu Thr Ile Ser Ser Leu Glu Ser

GAA GAT TTT GCA GAC TAT TAC TGT CTA CAA TAT ACT AAT TAT CCG CTC ACG
Glu Asp Phe Ala Asp Tyr Tyr Cys Leu Gln Tyr Thr Asn Tyr Pro Leu Thr

TTC GGT GCT GGG ACC AAG CTG GAG CTG AAA
Phe Gly Ala Gly Thr Lys Leu Glu Leu Lys
```

FIG. 1A

VH sequence of the heavy chain of murine 44H104 mab:

```
ATG GCT CTC CTG GTA CTG TTC CTC TCC CTG GCT GCA TTT CCA AGC TGT GGT
MET Ala Leu Leu Val Leu Phe Leu Ser Leu Ala Ala Phe Pro Ser Cys Gly

GTC CTG TCC CAG GTG CAG CTG AAG GAG TCA GGA CCT GGC CTG GTG GCG CCC
Val Leu Ser Gln Val Gln Leu Lys Glu Ser Gly Pro Gly Leu Val Ala Pro

TCA CAG AGC CTG TCC ATC ACT TGC ACT GTC TCT GGG TTT TCA TTA ACC AGC
Ser Gln Ser Leu Ser Ile Thr Cys Thr Val Ser Gly Phe Ser Leu Thr Ser

TAT GGT GTA CAC TGG GTT CGC CAG CCT CCA GGA AAG GGT CTG GAG TGG CTG
Tyr Gly Val His Trp Val Arg Gln Pro Pro Gly Lys Gly Leu Glu Trp Leu

GGA GTA ATA TGG GCT GGT GGA AGC ATA AAT TAT AAT TCG GCT CTC ATG TCC
Gly Val Ile Trp Ala Gly Gly Ser Ile Asn Tyr Asn Ser Ala Leu MET Ser

AGA CTG AGC ATC AGC AAA GAC AAC TTC AAG AGC CAA GTT TTC TTA AAA ATG
Arg Leu Ser Ile Ser Lys Asp Asn Phe Lys Ser Gln Val Phe Leu Lys MET

AGC AGT CTG CAA ACT GAT GAC ACA GCC ATG TAC TAC TGT GCC AGA GCC TAT
Ser Ser Leu Gln Thr Asp Asp Thr Ala MET Tyr Tyr Cys Ala Arg Ala Tyr

GGT GAC TAC GTC CAC TAT GCT ATG GAC TAC TGG GGT CAA GGA ACC TCA GTC
Gly Asp Tyr Val His Tyr Ala MET Asp Tyr Trp Gly Gln Gly Thr Ser Val

ACC GCC TCC TCA
Thr Ala Ser Ser
```

FIG. 1B

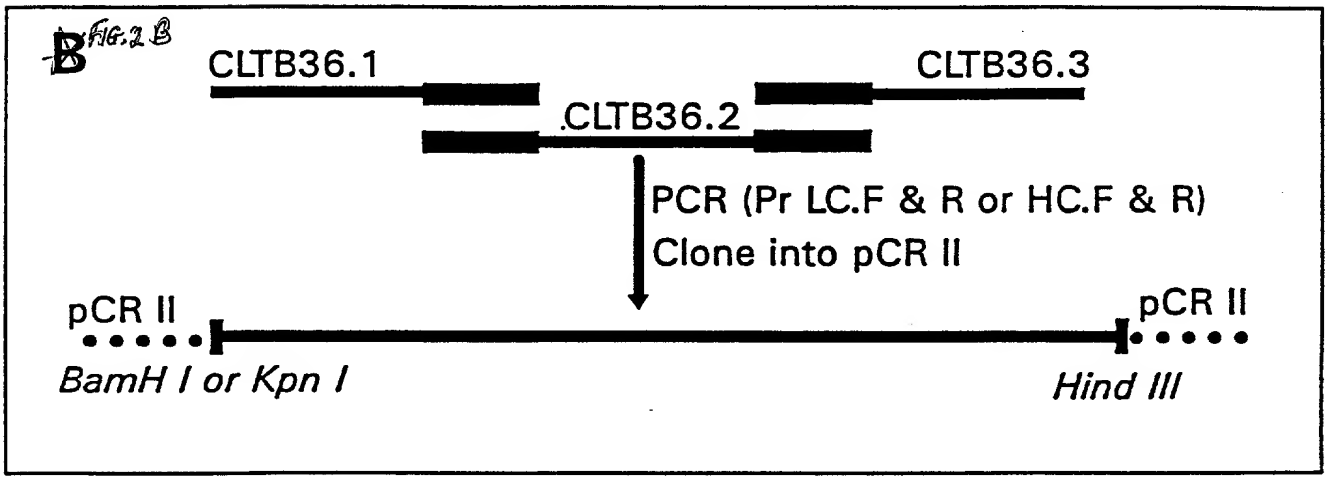
~~Figure 1~~ ~~Figure 1~~

Assembly of gene encoding CLTB36

A FIG. 2A

Amino acid seq. GPKEPFRDYVDRFYK NKRKRIHIGPGRAFYTTKN

Gene seq. GGTCCTAAAGAACCTTTTAGAGACTATGTTGATAGGTTTTA
TAAGAATAAGAGGAAGAGGATACATATAGGGCCTGGT
AGGGCTTTTTATACTACTAAGAATTAATAA



C FIG. 2C

CLTB36.1 CATTATGGATCCGGTCCTAAAGAACCTTTTAGAGACTATGTTGAT
AGGTTTTATAAGAAT

CLTB36.2 GCCCTACCAGGCCCTATATGTATCCTCTTCCTCTTATTCTTATAAA
ACCTA

CLTB36.3 AGGGCCTGGTAGGGCTTTTTATACTACTAAGAATTAATAAAAGCT
TTAGCG

BamH I

Pr LC.F CATTATGGATCCGGTCCTAA

Kpn I

Pr HC.F GTCAGGTACCGGTCCTAAAGAACCTTTTAG

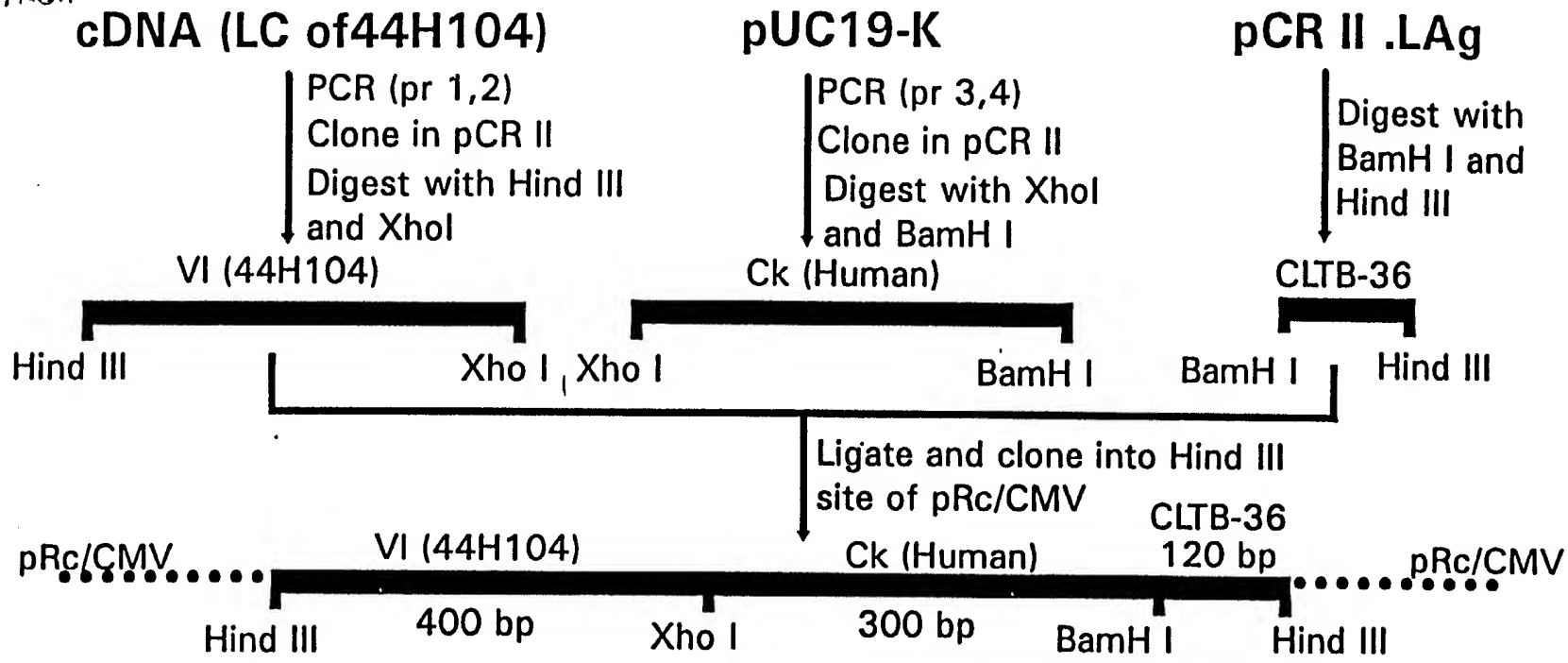
Hind III

Pr R GGCTAAAGCTTTTATTAATTC

Figure 2

Assembly of chimeric 44H104-CLTB36 light chain gene

A FIG3A



B FIG3B

Hind III

Pr. 1 AGCCTAAGCTTCCGCCATGGACATGAGGGTTCCTGCTC

Xho I

Pr. 2 CCGTTTCAGCTCGAGCTTGGTCCCAGCACCGAA

Xho I

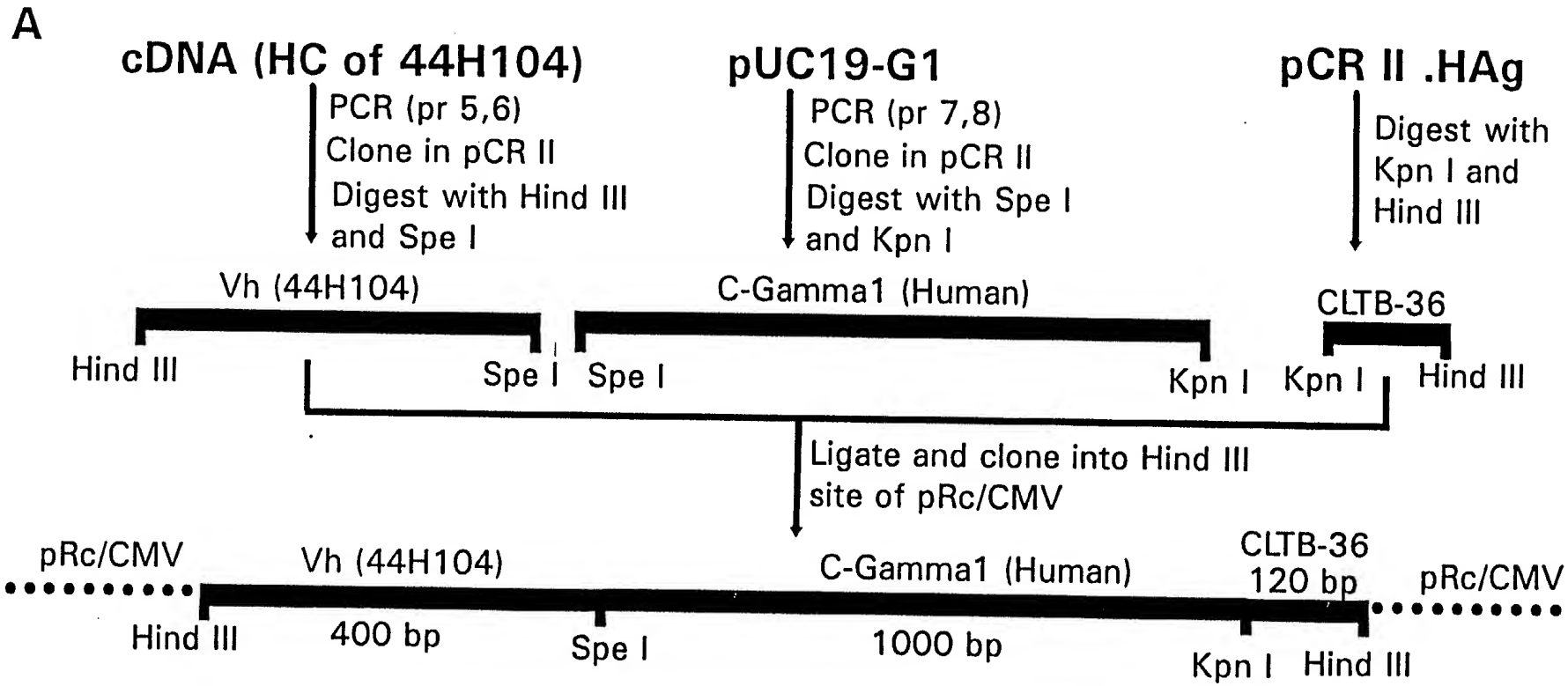
Pr. 3 CCTACTCGAGCTGAAACGGACTGTGGCTGCACCATCTGTC

BamH I

Pr. 4 ATTAAAGCTTTTACTAGGATCCACACTCTCCCCTGTTGAAGCTC

Figure 3

Assembly of chimeric 44H104-CLTB36 heavy chain gene



B

Hind III
Pr. 5 AGCTAAGCTTCCGCCATGGCTCTCCTGGTACTGTTC
Spe I
Pr. 6 GCGCACTAGTTCCTTGACCCCAGTAGTCC
Spe I
Pr. 7 GCGCACTAGTGTCACCGCCTCCTCAGCCTCCACCAAGGGCCCATCGGTCTTC
Hind III
Pr. 8 ACGCAAGCTTTTACTAGGTACCTTTACCCGGAGACAGGGAGAG

FIGURE 4

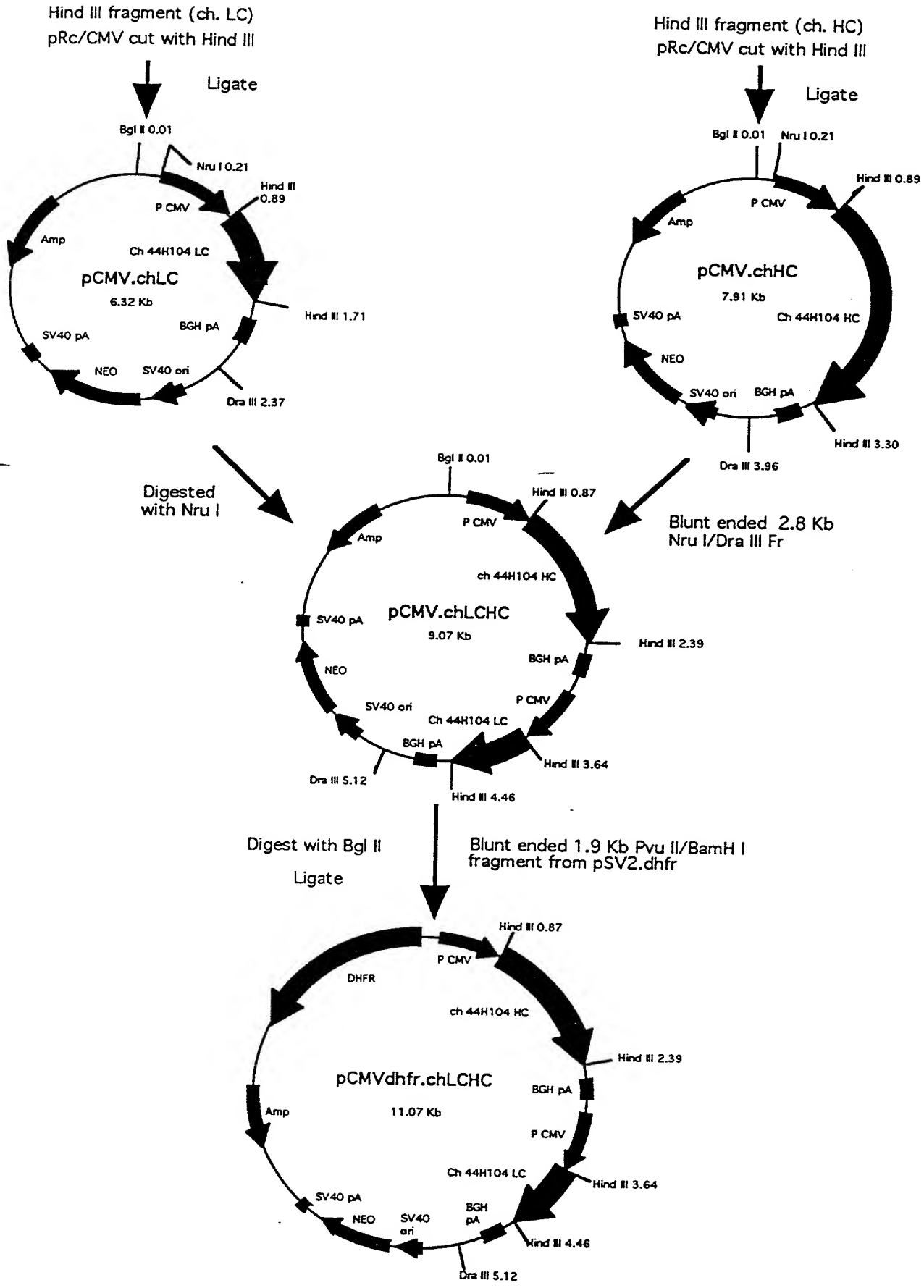

Hind III fragment (ch. LC)
pRc/CMV cut with Hind III
Ligate
Bgl II 0.01
Nru I 0.21
Hind III 0.89
P CMV
Amp
Ch 44H104 LC
pCMV.chLC
6.32 Kb
Hind III 1.71
BGH pA
SV40 pA
NEO
SV40 ori
Dra III 2.37
Hind III fragment (ch. HC)
pRc/CMV cut with Hind III
Ligate
Bgl II 0.01
Nru I 0.21
Hind III 0.89
P CMV
Amp
pCMV.chHC
7.91 Kb
SV40 pA
Ch 44H104 HC
NEO
SV40 ori
BGH pA
Hind III 3.30
Dra III 3.96
Digested
with Nru I
Blunt ended 2.8 Kb
Nru I/Dra III Fr
Bgl II 0.01
Hind III 0.87
P CMV
Amp
ch 44H104 HC
SV40 pA
pCMV.chLCHC
9.07 Kb
Hind III 2.39
BGH pA
NEO
SV40 ori
P CMV
Ch 44H104 LC
BGH pA
Hind III 3.64
Dra III 5.12
Hind III 4.46
Digest with Bgl II
Ligate
Blunt ended 1.9 Kb Pvu II/BamH I
fragment from pSV2.dhfr
Hind III 0.87
P CMV
DHFR
ch 44H104 HC
Hind III 2.39
pCMVdhfr.chLCHC
BGH pA
Amp
11.07 Kb
P CMV
Ch 44H104 LC
Hind III 3.64
SV40 pA
NEO
SV40
ori
BGH
pA
Hind III 4.46
Dra III 5.12

Figure 5

Epitope Recognition on Recombinant H^+L^+ Human Chimeric Antibody Bound to HLA-DR

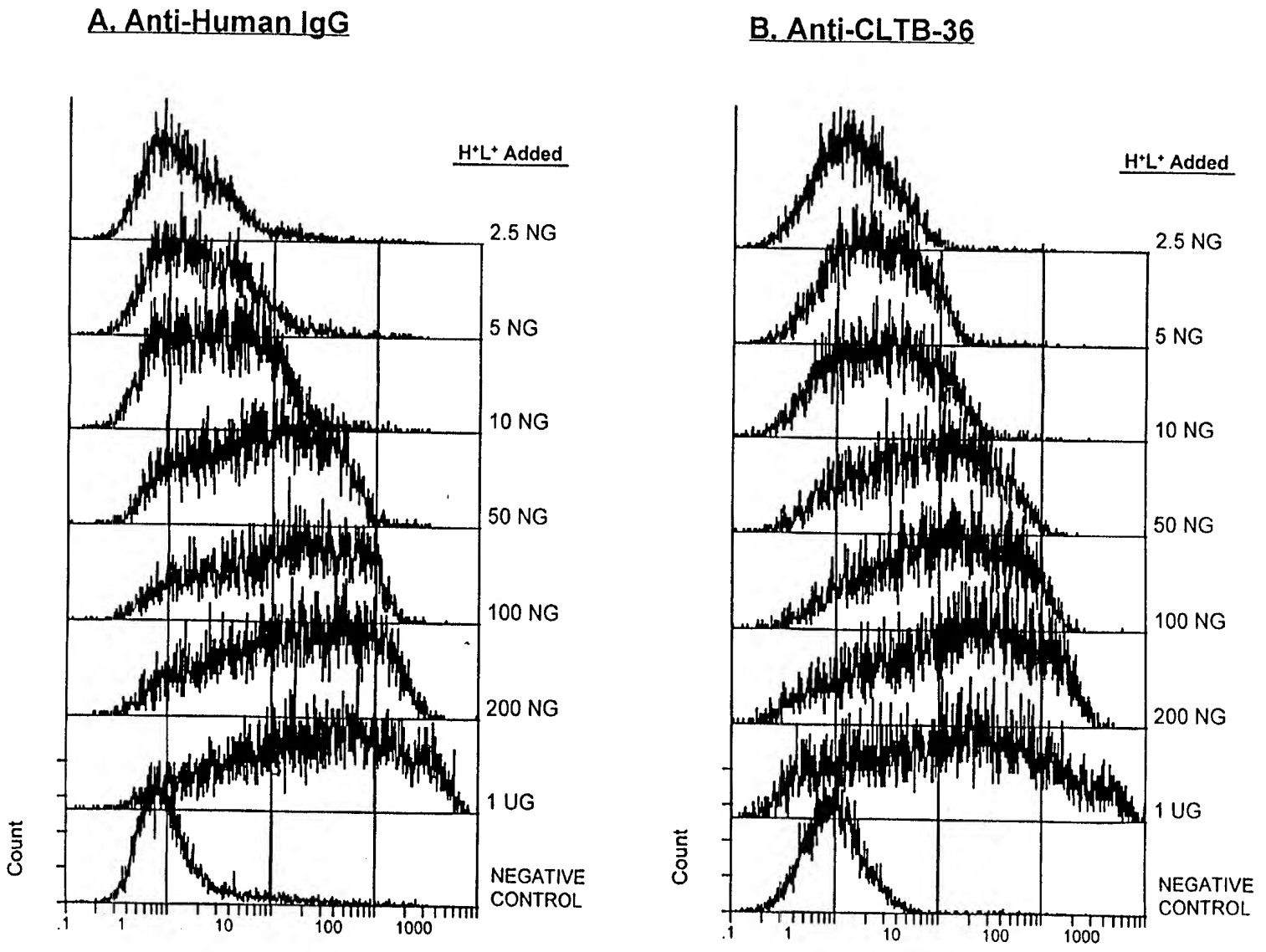
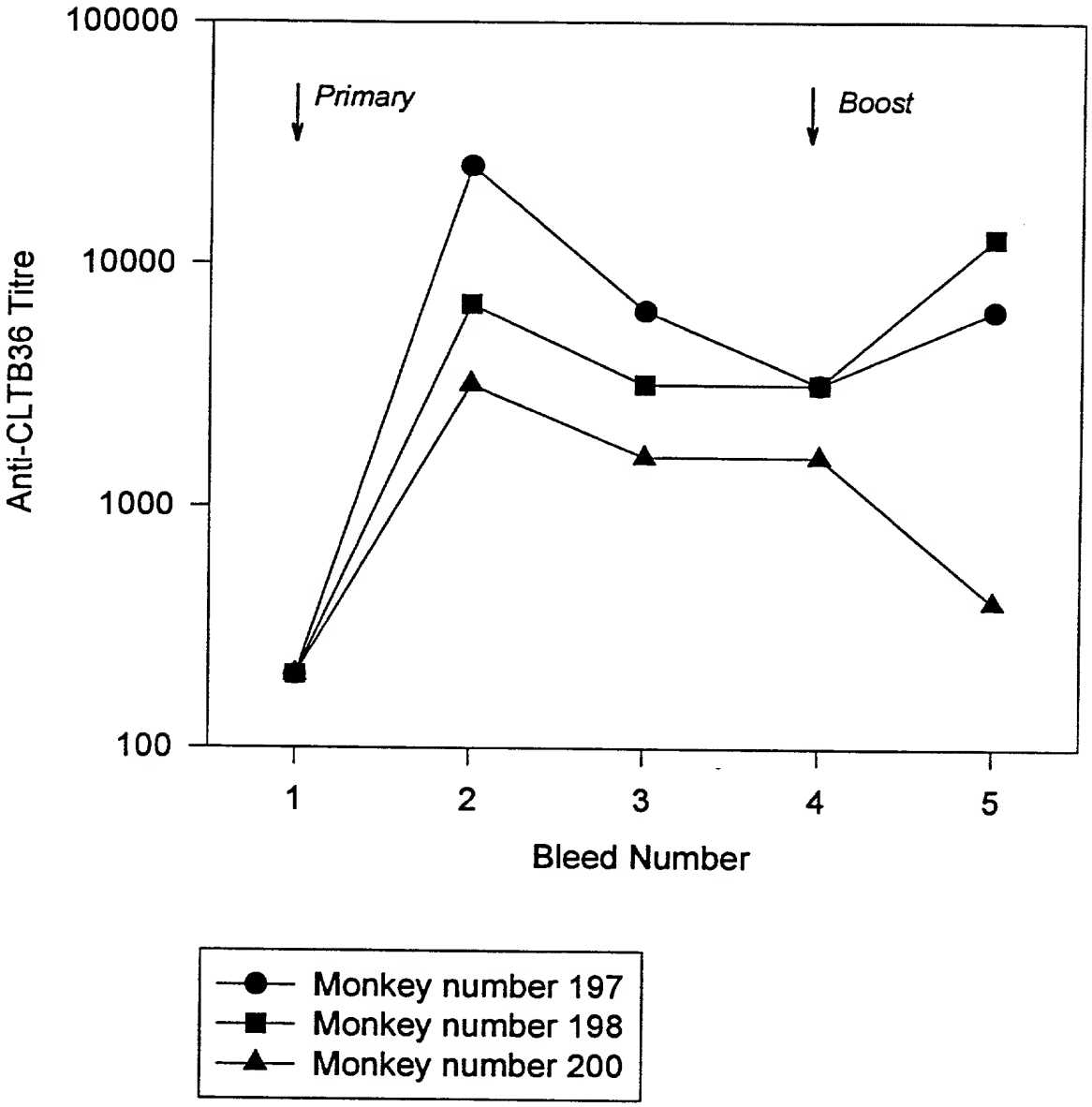


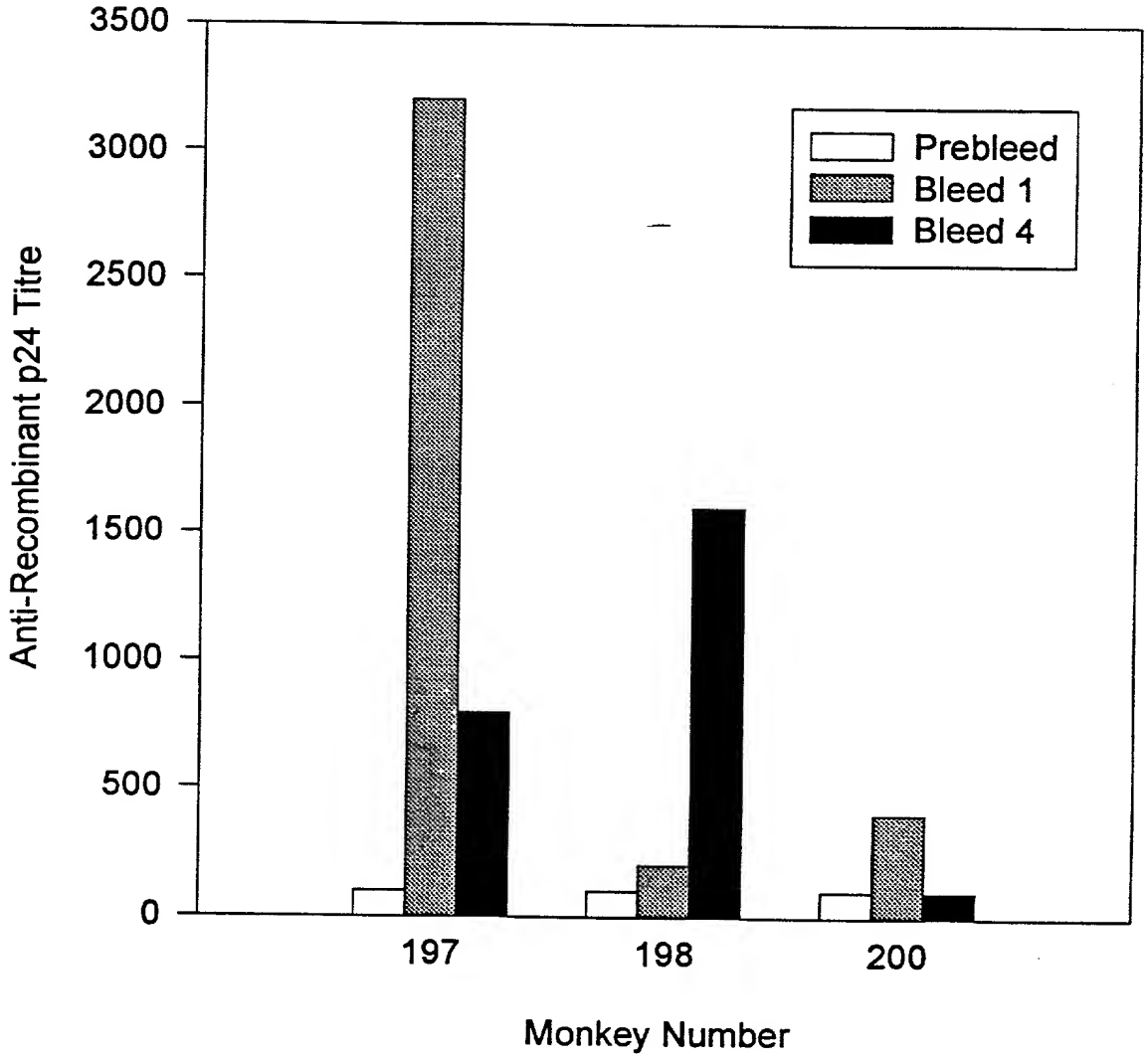
Figure 6.

Figure 9 - Anti-CLTB36 Titres

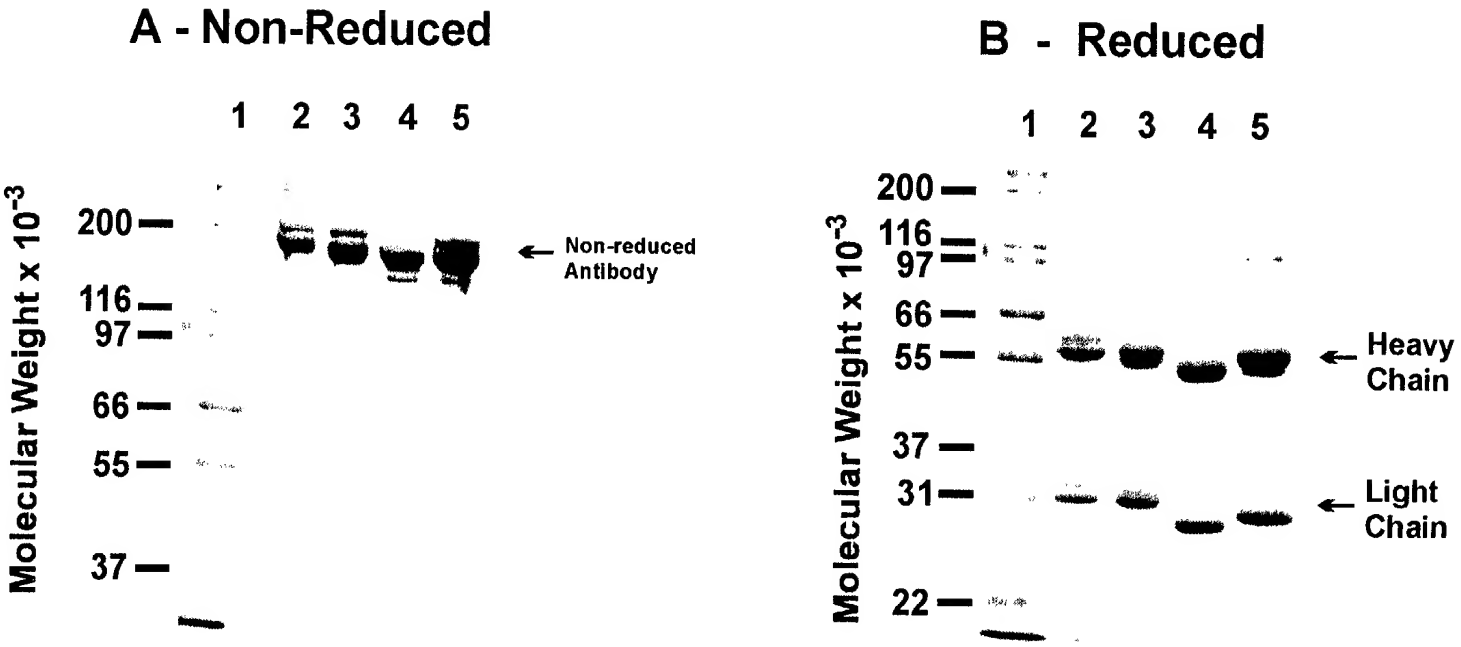


#8

Figure 8 - Anti-Recombinant p24 Titres



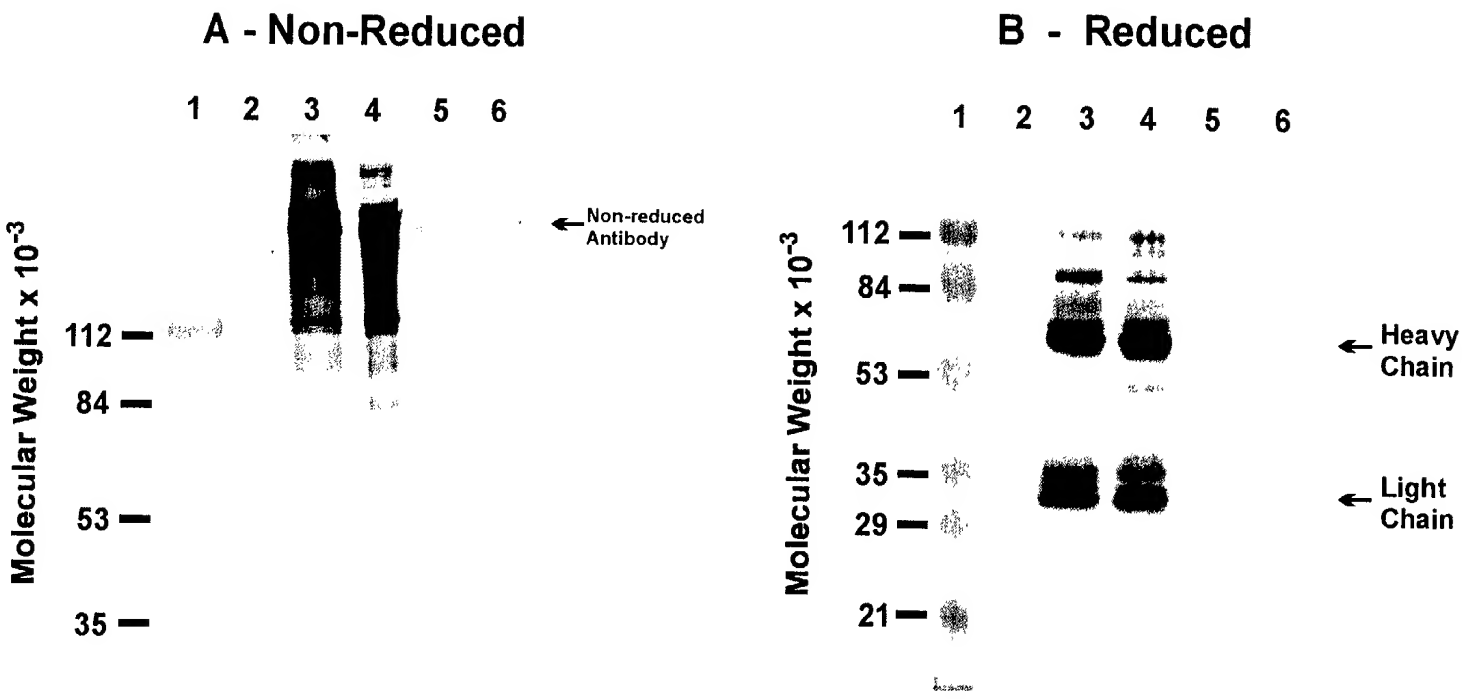
Coomassie Blue stained Polyacrylamide Gels of Recombinant Targeting Antibody



Lane 1 Molecular weight standards
Lane 2 Recombinant targeting antibody - Protein A purified
Lane 3 Recombinant targeting antibody - gel filtration purified
Lane 4 Mouse monoclonal antibody 44H104
Lane 5 Human IgG_1

FIG. 9

Western blots of recombinant targeting antibody probed with guinea pig anti-CLTB36 antiserum



Lane 1 Molecular weight standards
Lane 2 Blank lane
Lane 3 Recombinant targeting antibody - Protein A purified
Lane 4 Recombinant targeting antibody - gel filtration purified
Lane 5 Mouse monoclonal antibody 44H104
Lane 6 Human IgG_1

F10 10

OIPE

CRF Errors Corrected by the STIC Systems Branch

Serial Number: 09/007,093

CRF Processing Date: 2/20/98
Edited by: _____
Verified by: _____ (STIC staff)

☐ Changed a file from non-ASCII to ASCII

☐ Changed the margins in cases where the sequence text was "wrapped" down to the next line.

☐ Edited a format error in the Current Application Data section, specifically: _____

ENTERED

☐ Edited the Current Application Data section with the actual current number. The number inputted by the applicant was ☐ the prior application data; or ☐ other _____.

☐ Added the mandatory heading and subheadings for "Current Application Data".

☐ Edited the "Number of Sequences" field. The applicant spelled out a number instead of using an integer.

☑ Changed the spelling of a mandatory field (the headings or subheadings), specifically: "APLICATION" under (viii) PRIOR APP DATA

☐ Corrected the SEQ ID NO when obviously incorrect. The sequence numbers that were edited were: _____

☐ Inserted or corrected a nucleic number at the end of a nucleic line. SEQ ID NO's edited: _____

☐ Corrected subheading placement. All responses must be on the same line as each subheading. If the applicant placed a response below the subheading, this was moved to its appropriate place.

☐ Inserted colons after headings/subheadings. Headings edited included: _____

☐ Deleted extra, invalid, headings used by an applicant, specifically: _____

☐ Deleted: ☐ non-ASCII "garbage" at the beginning/end of files; ☐ secretary initials/filename at end of file; ☐ page numbers throughout text; ☐ other invalid text, such as _____.

☐ Inserted mandatory headings, specifically: _____

☐ Corrected an obvious error in the response, specifically: _____

☐ Edited identifiers where upper case is used but lower case is required, or vice versa.

☐ Corrected an error in the Number of Sequences field, specifically: _____

☐ A "Hard Page Break" code was inserted by the applicant. All occurrences had to be deleted.

☐ Deleted ***ending*** stop codon in amino acid sequences and adjusted the "(A)Length:" field accordingly (error due to a PatentIn bug). Sequences corrected: _____

☐ Other: _____

***Examiner: The above corrections must be communicated to the applicant in the first Office Action. DO NOT send a copy of this form.**

3/1/95

This Raw Listing contains the General Information Section and up to the first 5 pages.

SEQUENCE LISTING

(1) General Information:

(i) APPLICANT: Anand, Naveen N
Barber, Brian H
Cates, George A
Caterini, Judith E
Klein, Michel H

(ii) TITLE OF INVENTION: CHIMERIC ANTIBODIES FOR DELIVERY OF ANTIGENS TO SELECTED CELLS OF THE IMMUNE SYSTEM

(iii) NUMBER OF SEQUENCES: 20

(iv) CORRESPONDENCE ADDRESS:
(A) ADDRESSEE: Sim & McBurney
(B) STREET: Suite 701, 330 University Avenue
(C) CITY: Toronto
(D) STATE: Ontario
(E) COUNTRY: Canada
(F) ZIP: M5G 1R7

(v) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: PatentIn Release #1.0, Version #1.30

(vi) CURRENT APPLICATION DATA:
(A) APPLICATION NUMBER:
(B) FILING DATE:
(C) CLASSIFICATION:

(vii) PRIOR APPLICATION DATA:
(A) APPLICATION NUMBER: US 08/483,576
(B) FILING DATE: 07-JUN-1995

(viii) ATTORNEY/AGENT INFORMATION:
(A) NAME: Stewart, Michael I
(B) REGISTRATION NUMBER: 24,973
(C) REFERENCE/DOCKET NUMBER: 1038-765

(ix) TELECOMMUNICATION INFORMATION:
(A) TELEPHONE: (416) 595-1155
(B) TELEFAX: (416) 595-1163

(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 387 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

ATGGACATGA GGGTTCCTGC TCACGTTTTT GGCTTCTTGT TGCTCTGGTT TCCAGGTACC 60

AGATGTGACA TCCAGATGAC CCAGTCTCCA TCCTCCTTAT CTGCCTCTCT GGGACAAAGA 120

GTCAGTCTCA CTTGTCGGGC AAGTCAGGAA ATTAGTGGTT ACTTAACCTG GCTTCAGCAG 180

AAACCAGATG GAACTATTAA ACGCCTGGTC TACGCCGCGT CCACTTTAGA TTCTGGTGTC 240

CCAAAAAGGT TCAGTGGCAG TAGGTCTGGG TCAGATTATT CTCTCACCAT CAGCAGCCTT 300

GAGTCTGAAG ATTTTGCAGA CTATTACTGT CTACAATATA CTAATTATCC GCTCACGTTC 360

GGTGCTGGGA CCAAGCTGGA GCTGAAA 387

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 129 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

Met Asp Met Arg Val Pro Ala His Val Phe Gly Phe Leu Leu Leu Trp
1 5 10 15

Phe Pro Gly Thr Arg Cys Asp Ile Gln Met Thr Gln Ser Pro Ser Ser
20 25 30

Leu Ser Ala Ser Leu Gly Gln Arg Val Ser Leu Thr Cys Arg Ala Ser
35 40 45

Gln Glu Ile Ser Gly Tyr Leu Thr Trp Leu Gln Gln Lys Pro Asp Gly
50 55 60

Thr Ile Lys Arg Leu Val Tyr Ala Ala Ser Thr Leu Asp Ser Gly Val
65 70 75 80

Pro Lys Arg Phe Ser Gly Ser Arg Ser Gly Ser Asp Thr Ser Leu Thr
85 90 95

Ile Ser Ser Leu Glu Ser Glu Asp Phe Ala Asp Tyr Tyr Cys Leu Gln
100 105 110

Tyr Thr Asn Tyr Pro Leu Thr Phe Gly Ala Gly Thr Lys Leu Glu Leu
115 120 125

Lys

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 420 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

ATGGCTCTCC TGGTACTGTT CCTCTCCCTG GCTGCATTTC CAAGCTGTGG TGTCCTGTCC 60

CAGGTGCAGC TGAAGGAGTC AGGACCTGGC CTGGTGGCGC CCTCACAGAG CCTGTCCATC 120

ACTTGCACTG TCTCTGGGTT TCATTAACC AGCTATGGTG TACACTGGGT TCGCCAGCCT 180

CCAGGAAAGG GTCTGGAGTG GCTGGGAGTA ATATGGGCTG GTGGAAGCAT AAATTATAAT 240

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GACTACGTCC ACTATGCTAT GGACTACTGG GGTCAAGGAA CCTCAGTCAC CGCCTCCTCA 420

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 140 amino acids
(B) TYPE: amino acid

(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

```
Met Ala Leu Leu Val Leu Phe Leu Ser Leu Ala Ala Phe Pro Ser Cys
1               5                   10                  15

Gly Val Leu Ser Gln Val Gln Leu Lys Glu Ser Gly Pro Gly Leu Val
            20                  25                  30

Ala Pro Ser Gln Ser Leu Ser Ile Thr Cys Thr Val Ser Gly Phe Ser
        35                  40                  45

Leu Thr Ser Tyr Gly Val His Trp Val Arg Gln Pro Pro Gly Lys Gly
    50                  55                  60

Leu Glu Trp Leu Gly Val Ile Trp Ala Gly Gly Ser Ile Asn Tyr Asn
65                  70                  75                  80

Ser Ala Leu Met Ser Arg Leu Ser Ile Ser Lys Asp Asn Phe Lys Ser
                85                  90                  95

Gln Val Phe Leu Lys Met Ser Ser Leu Gln Thr Asp Asp Thr Ala Met
            100                 105                 110

Tyr Tyr Cys Ala Arg Ala Tyr Gly Asp Tyr Val His Tyr Ala Met Asp
        115                 120                 125

Tyr Trp Gly Gln Gly Thr Ser Val Thr Ala Ser Ser
    130                 135                 140
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(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 34 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

```
Gly Pro Lys Glu Pro Phe Arg Asp Tyr Val Asp Arg Phe Tyr Lys Asn
1               5                   10                  15
```

Lys Arg Lys Arg Ile His Ile Gly Pro Gly Arg Ala Phe Tyr Thr Thr
20 25 30

Lys Asn

(2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 108 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

GGTCCTAAAG AACCTTTTAG AGACTATGTT GATAGGTTTT ATAAGAATAA GAGGAAGAGG 60

ATACATATAG GGCCTGGTAG GGCTTTTTAT ACTACTAAGA ATTAATAA 108

(2) INFORMATION FOR SEQ ID NO:7:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 60 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

CATTATGGAT CCGGTCCTAA AGAACCTTTT AGAGACTATG TTGATAGGTT TTATAAGAAT 60

(2) INFORMATION FOR SEQ ID NO:8:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 51 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

SEQUENCE VERIFICATION REPORT
PATENT APPLICATION ***US/09/007,093***

INPUT SET: S23619.raw

Line	Error	Original Text

This Raw Listing contains the General Information Section and up to the first 5 pages.

Does Not Comply
Corrected Diskette Needed

SEQUENCE LISTING

(1) General Information:

(i) APPLICANT: Anand, Naveen N
Barber, Brian H
Cates, George A
Caterini, Judith E
Klein, Michel H

(ii) TITLE OF INVENTION: CHIMERIC ANTIBODIES FOR DELIVERY OF ANTIGENS TO SELECTED CELLS OF THE IMMUNE SYSTEM

(iii) NUMBER OF SEQUENCES: 20

(iv) CORRESPONDENCE ADDRESS:
(A) ADDRESSEE: Sim & McBurney
(B) STREET: Suite 701, 330 University Avenue
(C) CITY: Toronto
(D) STATE: Ontario
(E) COUNTRY: Canada
(F) ZIP: M5G 1R7

(v) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: PatentIn Release #1.0, Version #1.30

(vi) CURRENT APPLICATION DATA:
(A) APPLICATION NUMBER:
(B) FILING DATE:
(C) CLASSIFICATION:

--> (vii) PRIOR APLICATION DATA:
--> (A) APPLICATION NUMBER: US 08/483,576
(B) FILING DATE: 07-JUN-1995
~~(C) CLASSIFICATION:~~

(viii) ATTORNEY/AGENT INFORMATION:
(A) NAME: Stewart, Michael I
(B) REGISTRATION NUMBER: 24,973
(C) REFERENCE/DOCKET NUMBER: 1038-765

(ix) TELECOMMUNICATION INFORMATION:
(A) TELEPHONE: (416) 595-1155

(B) TELEFAX: (416) 595-1163

(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 387 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

ATGGACATGA GGGTTCCTGC TCACGTTTTT GGCTTCTTGT TGCTCTGGTT TCCAGGTACC 60

AGATGTGACA TCCAGATGAC CCAGTCTCCA TCCTCCTTAT CTGCCTCTCT GGGACAAAGA 120

GTCAGTCTCA CTTGTCGGGC AAGTCAGGAA ATTAGTGGTT ACTTAACCTG GCTTCAGCAG 180

AAACCAGATG GAACTATTAA ACGCCTGGTC TACGCCGCGT CCACTTTAGA TTCTGGTGTC 240

CCAAAAAGGT TCAGTGGCAG TAGGTCTGGG TCAGATTATT CTCTCACCAT CAGCAGCCTT 300

GAGTCTGAAG ATTTTGCAGA CTATTACTGT CTACAATATA CTAATTATCC GCTCACGTTC 360

GGTGCTGGGA CCAAGCTGGA GCTGAAA 387

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 129 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

```
Met Asp Met Arg Val Pro Ala His Val Phe Gly Phe Leu Leu Leu Trp
1               5                  10                  15

Phe Pro Gly Thr Arg Cys Asp Ile Gln Met Thr Gln Ser Pro Ser Ser
            20                  25                  30

Leu Ser Ala Ser Leu Gly Gln Arg Val Ser Leu Thr Cys Arg Ala Ser
        35                  40                  45
```

```
100
101       Gln Glu Ile Ser Gly Tyr Leu Thr Trp Leu Gln Gln Lys Pro Asp Gly
102           50                  55                  60
103
104       Thr Ile Lys Arg Leu Val Tyr Ala Ala Ser Thr Leu Asp Ser Gly Val
105       65                  70                  75                  80
106
107       Pro Lys Arg Phe Ser Gly Ser Arg Ser Gly Ser Asp Thr Ser Leu Thr
108                       85                  90                  95
109
110       Ile Ser Ser Leu Glu Ser Glu Asp Phe Ala Asp Tyr Tyr Cys Leu Gln
111                   100                 105                 110
112
113       Tyr Thr Asn Tyr Pro Leu Thr Phe Gly Ala Gly Thr Lys Leu Glu Leu
114               115                 120                 125
115
116       Lys
117
118
119
120   (2) INFORMATION FOR SEQ ID NO:3:
121
122        (i) SEQUENCE CHARACTERISTICS:
123             (A) LENGTH: 420 base pairs
124             (B) TYPE: nucleic acid
125             (C) STRANDEDNESS: single
126             (D) TOPOLOGY: linear
127
128
129
130
131
132        (xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:
133
134   ATGGCTCTCC TGGTACTGTT CCTCTCCCTG GCTGCATTTC CAAGCTGTGG TGTCCTGTCC      60
135
136   CAGGTGCAGC TGAAGGAGTC AGGACCTGGC CTGGTGGCGC CCTCACAGAG CCTGTCCATC     120
137
138   ACTTGCACTG TCTCTGGGTT TTCATTAACC AGCTATGGTG TACACTGGGT TCGCCAGCCT     180
139
140   CCAGGAAAGG GTCTGGAGTG GCTGGGAGTA ATATGGGCTG GTGGAAGCAT AAATTATAAT     240
141
142   TCGGCTCTCA TGTCCAGACT GAGCATCAGC AAAGACAACT TCAAGAGCCA AGTTTTCTTA     300
143
144   AAAATGAGCA GTCTGCAAAC TGATGACACA GCCATGTACT ACTGTGCCAG AGCCTATGGT     360
145
146   GACTACGTCC ACTATGCTAT GGACTACTGG GGTCAAGGAA CCTCAGTCAC CGCCTCCTCA     420
147
148
149   (2) INFORMATION FOR SEQ ID NO:4:
150
151        (i) SEQUENCE CHARACTERISTICS:
152             (A) LENGTH: 140 amino acids
```

(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

```
Met Ala Leu Leu Val Leu Phe Leu Ser Leu Ala Ala Phe Pro Ser Cys
1               5                  10                  15

Gly Val Leu Ser Gln Val Gln Leu Lys Glu Ser Gly Pro Gly Leu Val
            20                  25                  30

Ala Pro Ser Gln Ser Leu Ser Ile Thr Cys Thr Val Ser Gly Phe Ser
        35                  40                  45

Leu Thr Ser Tyr Gly Val His Trp Val Arg Gln Pro Pro Gly Lys Gly
    50                  55                  60

Leu Glu Trp Leu Gly Val Ile Trp Ala Gly Gly Ser Ile Asn Tyr Asn
65                  70                  75                  80

Ser Ala Leu Met Ser Arg Leu Ser Ile Ser Lys Asp Asn Phe Lys Ser
                85                  90                  95

Gln Val Phe Leu Lys Met Ser Ser Leu Gln Thr Asp Asp Thr Ala Met
            100                 105                 110

Tyr Tyr Cys Ala Arg Ala Tyr Gly Asp Tyr Val His Tyr Ala Met Asp
        115                 120                 125

Tyr Trp Gly Gln Gly Thr Ser Val Thr Ala Ser Ser
    130                 135                 140
```

(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 34 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

```
Gly Pro Lys Glu Pro Phe Arg Asp Tyr Val Asp Arg Phe Tyr Lys Asn
1               5                  10                  15
```

Lys Arg Lys Arg Ile His Ile Gly Pro Gly Arg Ala Phe Tyr Thr Thr
20 25 30

Lys Asn

(2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 108 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

GGTCCTAAAG AACCTTTTAG AGACTATGTT GATAGGTTTT ATAAGAATAA GAGGAAGAGG 60

ATACATATAG GGCCTGGTAG GGCTTTTTAT ACTACTAAGA ATTAATAA 108

(2) INFORMATION FOR SEQ ID NO:7:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 60 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

CATTATGGAT CCGGTCCTAA AGAACCTTTT AGAGACTATG TTGATAGGTT TTATAAGAAT 60

(2) INFORMATION FOR SEQ ID NO:8:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 51 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

SEQUENCE VERIFICATION REPORT
PATENT APPLICATION *US/09/007,093*

DATE: 02/20/98
TIME: 12:15:55

INPUT SET: S23619.raw

Line	Error	Original Text
35	Unknown or Misplaced Identifier	(vii) PRIOR APLICATION DATA:
36	Wrong application Serial Number	(A) APPLICATION NUMBER: US 08/483,576